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December, 1961

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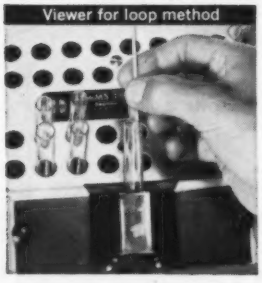
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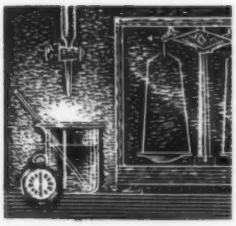
a glance at yesterday in relation to today



IN DECEMBER—(1905)—A preliminary communication to the Chemical Society (London) discusses a method of estimating tyrosine by bromination and the use of this estimate as a measure of proteolytic activity. Tyrosine is reported to be an early product of tryptic hydrolysis, all the tyrosine in a protein being liberated during the first stage of tryptic digestion. Resistance of the protein tyrosine nucleus to *peptic* hydrolysis is confirmed. This appears to offer a trustworthy method of differentiating peptic from tryptic enzymes.[1]

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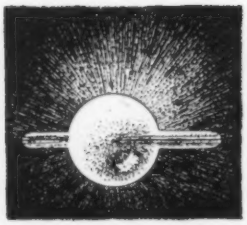

IN DECEMBER—(1934)—*Science*[2] reviews some of the year's important advances in physics and chemistry. By bombarding boron, magnesium, and aluminum with alpha particles, F. Joliot and Irene Curie-Joliot created artificial radioactivity for the first time. They predict the development of new radioisotopes useful in medicine. Triple-weight hydrogen, three times as heavy as the ordinary kind, was discovered by Rutherford, Oliphant, and Hartek at Cambridge; Tuve, Hafstad, and Dahl at the Carnegie Institute; and Harnwell, Smyth, Bleakney, and Smith at Princeton.

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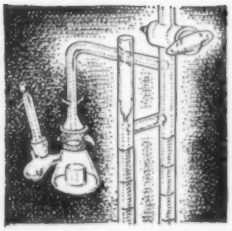

IN DECEMBER—(1954)—Brawerman and Chargaff[3] report some studies on malt, liver, and prostate nucleoside phosphotransferases (enzymes which transfer organically esterified phosphate to nucleosides). The prostate enzyme differentiates between ribo- and deoxyribosides, the liver enzyme between uracil and cytosine ribosides. The malt enzyme accepts phosphate from many nucleotides and may function as a catalyst for nucleotide interconversion—a role analagous to that of the transaminases in amino acid metabolism.

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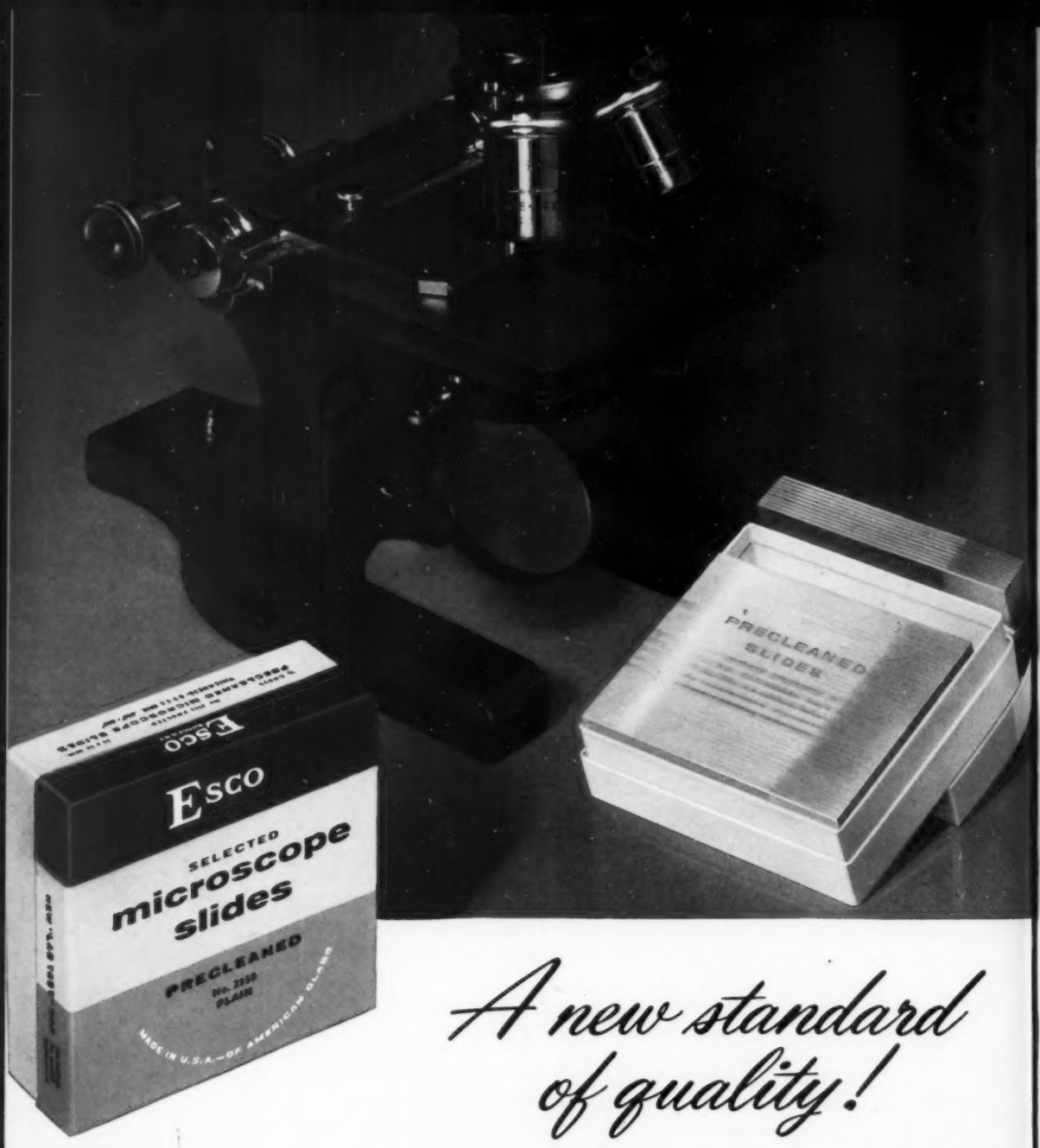

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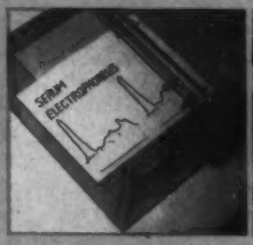

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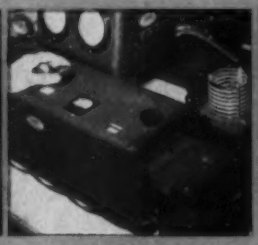

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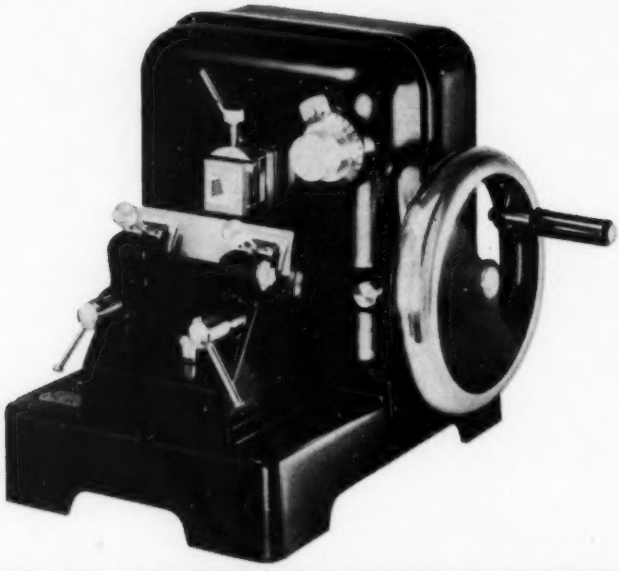

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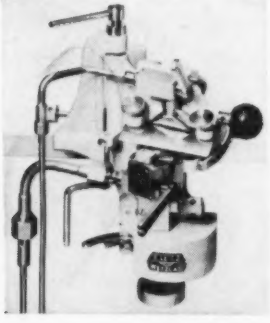

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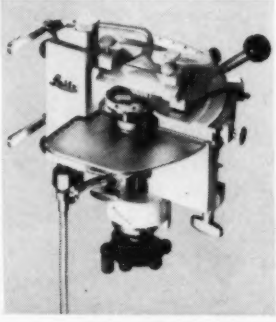

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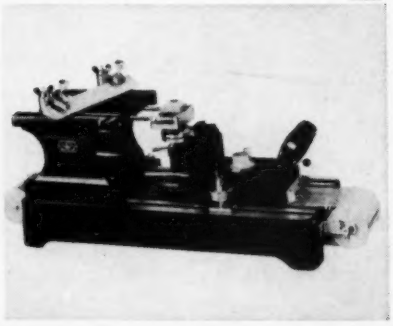

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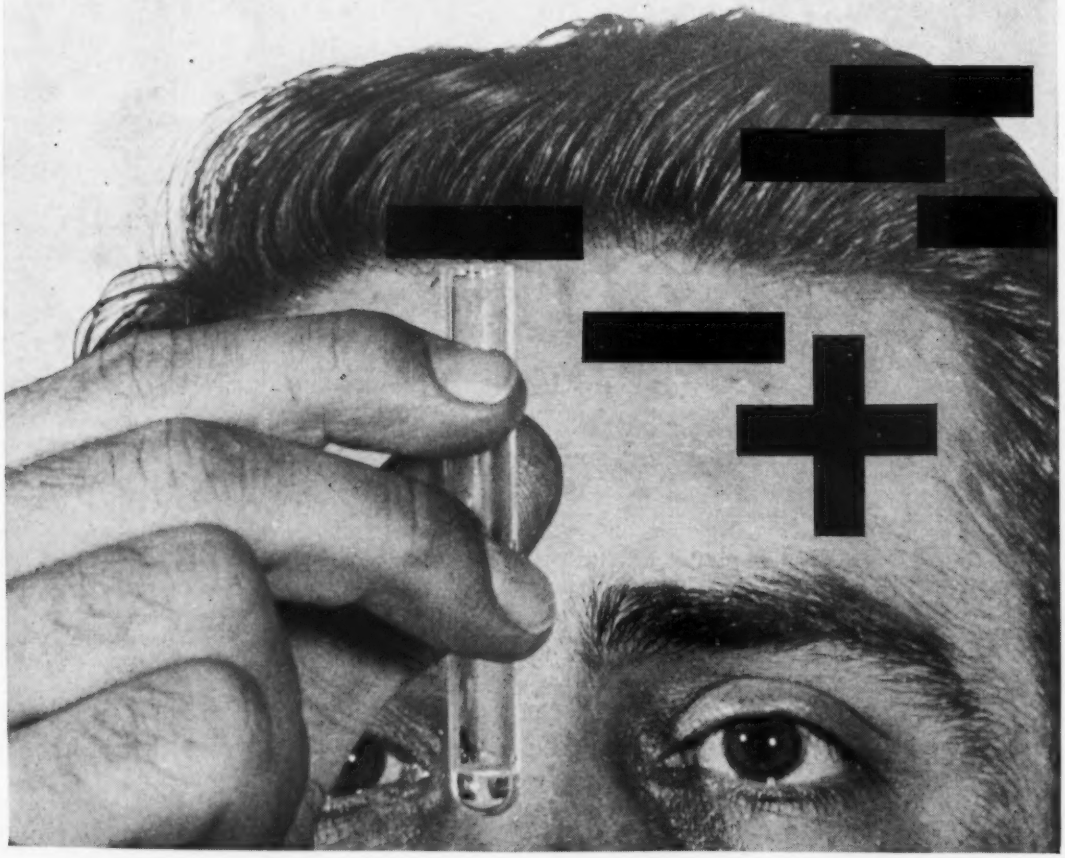

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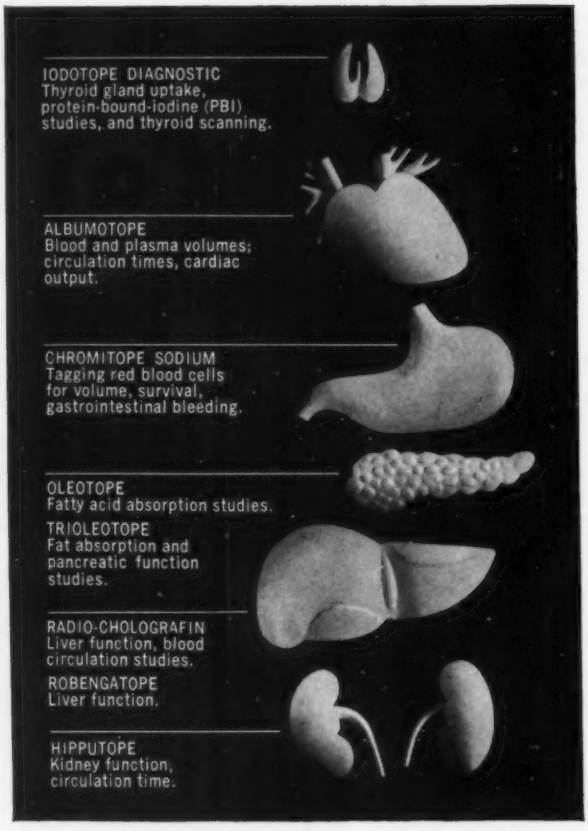

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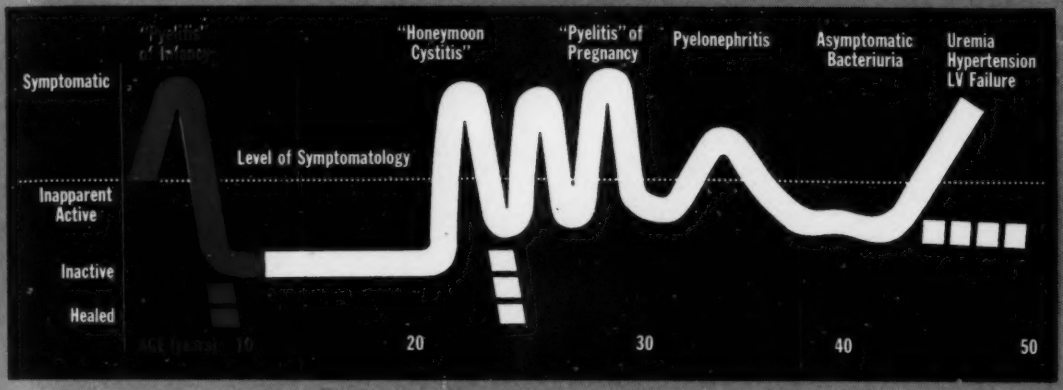

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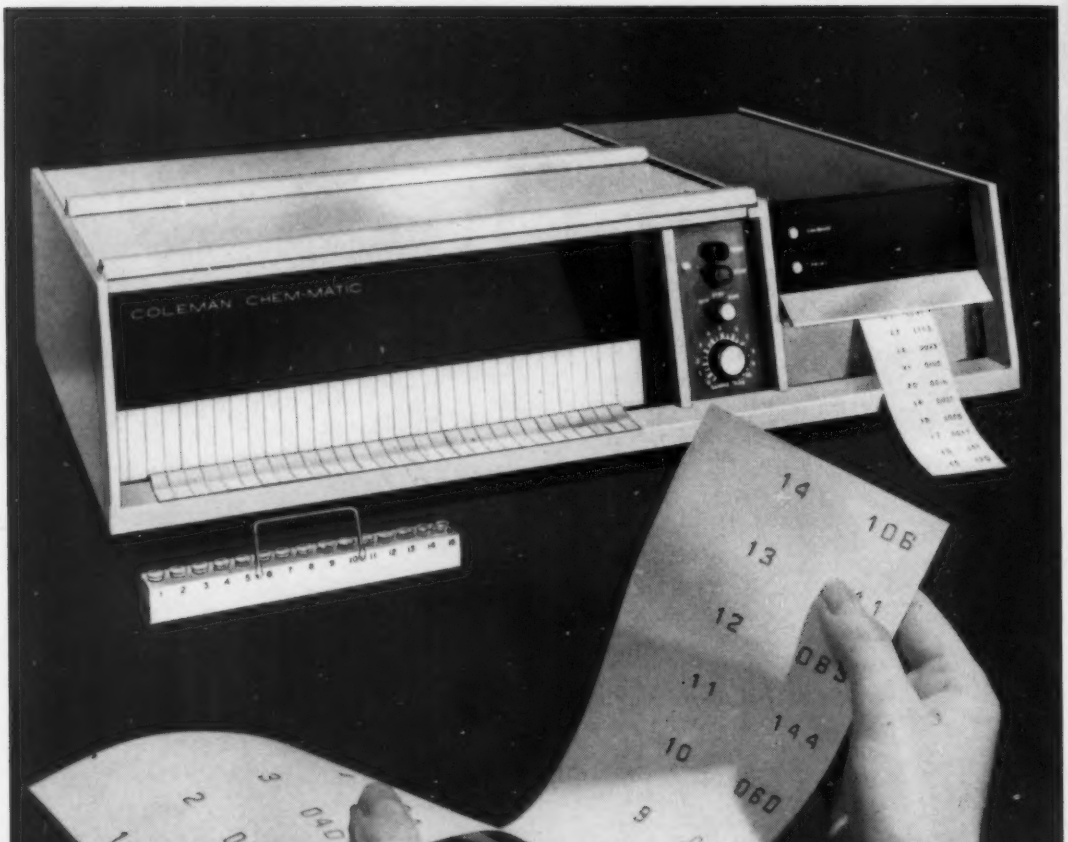

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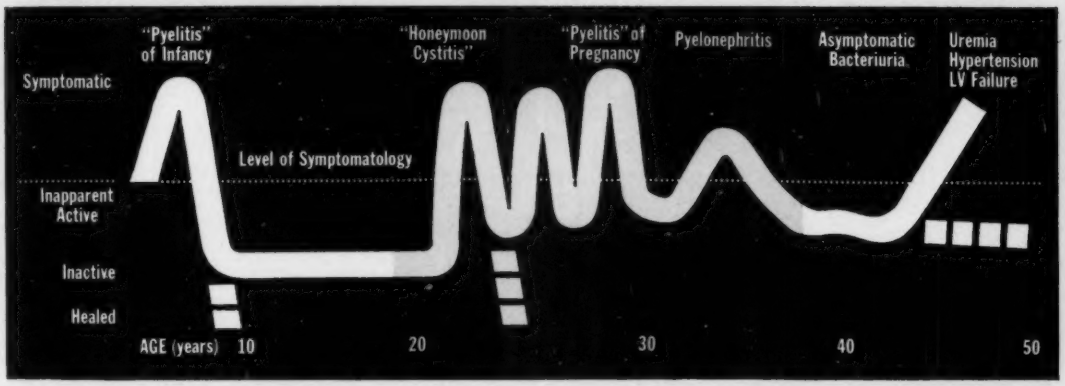
Natural History of Pyelonephritis



". . . the theme that runs through the carefully taken history of most uremic patients with chronic pyelonephritis—the burning on urination of infancy, the chills and fever in childhood, the 'honeymoon' pyelitis, the recurrent urethritis treated so well and often locally—and yet the termination in uremia."[1]

the child-bearing age—a second major stage for urinary tract infection

"The fact that the many cases of chronic and finally, lethal, upper urinary infections in women begin or recur during gestation is especially challenging."[2] "We now believe that all prepartum women should have one quantitative urine culture as part of their medical management."[3]

Furadantin—when pregnancy initiates (or activates) urinary tract infection

In a study of 104 pregnant women with urinary tract infections: "FURADANTIN was highly effective in the treatment of these infections during all stages . . . and frequently offers the best chance of effecting a clinical cure."[4]

FURADANTIN DOSAGE DURING PREGNANCY AND THE PUERPERIUM: The average dose is one 100 mg. tablet 4 times daily, given with meals and with food or milk on retiring, to prevent nausea. For acute, uncomplicated infections, 50 mg. q.i.d. may be administered. If improvement does not occur in 2 or 3 days, increase dosage to 100 mg. q.i.d.

SUPPLIED: Tablets, 50 mg. and 100 mg. Oral Suspension, 25 mg. per 5 cc. tsp.

REFERENCES: 1. Birchall, R.: Am. Practit. *11*:918, 1960. 2. Benson, R. C., and Mitchell, J. C.: Clin. Obstet. Gynec. *1*:97, 1958. 3. Favour, C. B.: Southern Med. J. *54*:848, 1961. 4. Nesbitt, R. E. L., Jr., and Young, J. E.: Obstet. Gynec. (N. Y.) *10*:89, 1957.

Complete information in package insert or on request to the Medical Director.

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To cure or control infection throughout the urinary system at every age of life... at every stage of infection

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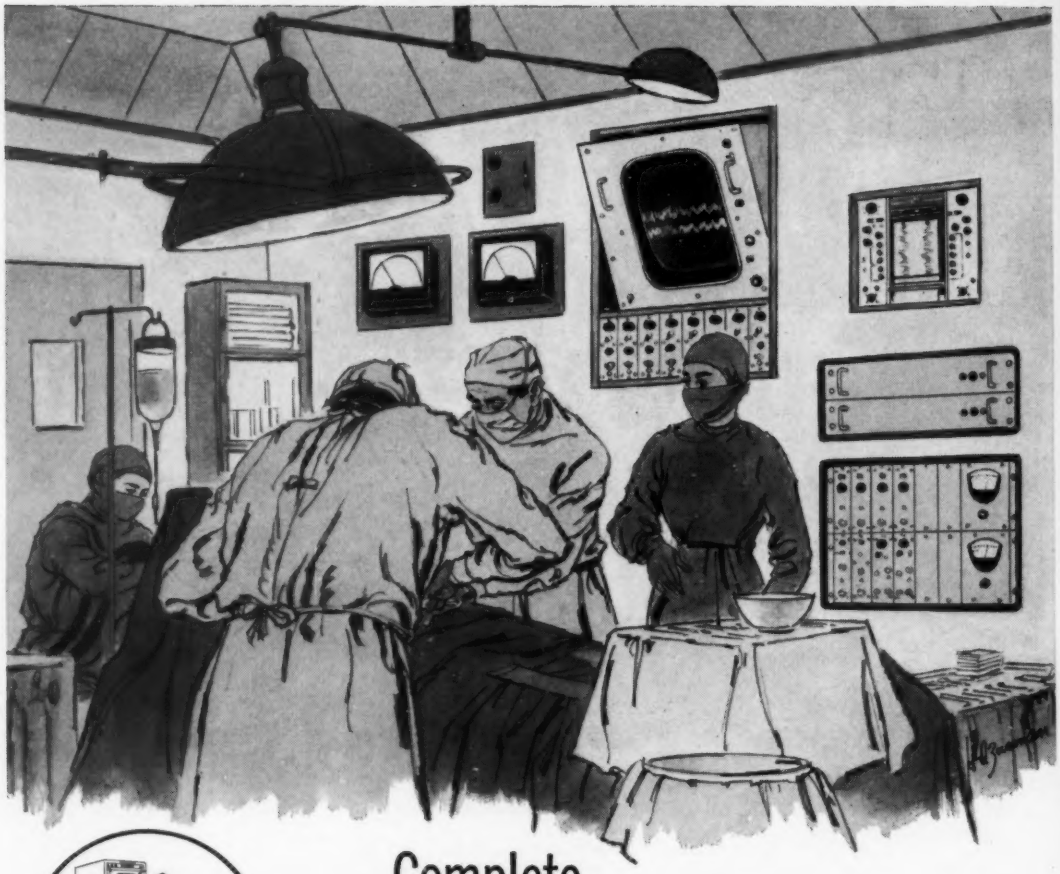

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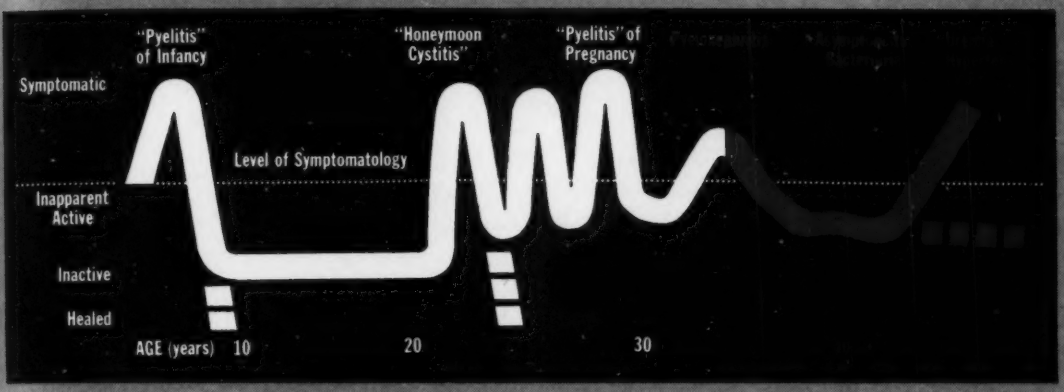
Natural History of Pyelonephritis



"... the theme that runs through the carefully taken history of most uremic patients with chronic pyelonephritis—the burning on urination of infancy, the chills and fever in childhood, the 'honeymoon' pyelitis, the recurrent urethritis treated so well and often locally—and yet the termination in uremia."[1]

during the middle and later years—relapse, reinfection, renal failure

"... the physician treating a patient with established chronic urinary tract infection faces a grave problem of management."[2]

Furadantin—to preserve function; to prolong life

"... certain patients with renal insufficiency derived measurable benefit from prolonged nitrofurantoin treatment; as infection was suppressed their renal function improved. This effect was sufficiently pronounced to be considered an important component of the management of uremia accompanying chronic pyelonephritis."[2]

FURADANTIN DOSAGE IN LONG-TERM THERAPY: "With normal renal function, the dosage schedule of 50 mg. four times daily in adults gave urinary nitrofurantoin concentrations that usually exceeded 5 mg. per 100 mg. throughout the day. This level was thought to be sufficient, on the basis of bacterial sensitivity determinations."[3] In refractory cases, 100 mg. q.i.d. daily is recommended.

SUPPLIED: Tablets, 50 mg. and 100 mg. Oral Suspension, 25 mg. per 5 cc. tsp.

REFERENCES: 1. Birchall, R.: Am. Practit. *11*:918, 1960. 2. Jawetz, E., et al.: A.M.A. Arch. Intern. Med. *100*:549, 1957. 3. Lippman, R. W., et al.: J. Urol. *80*:77, 1958.

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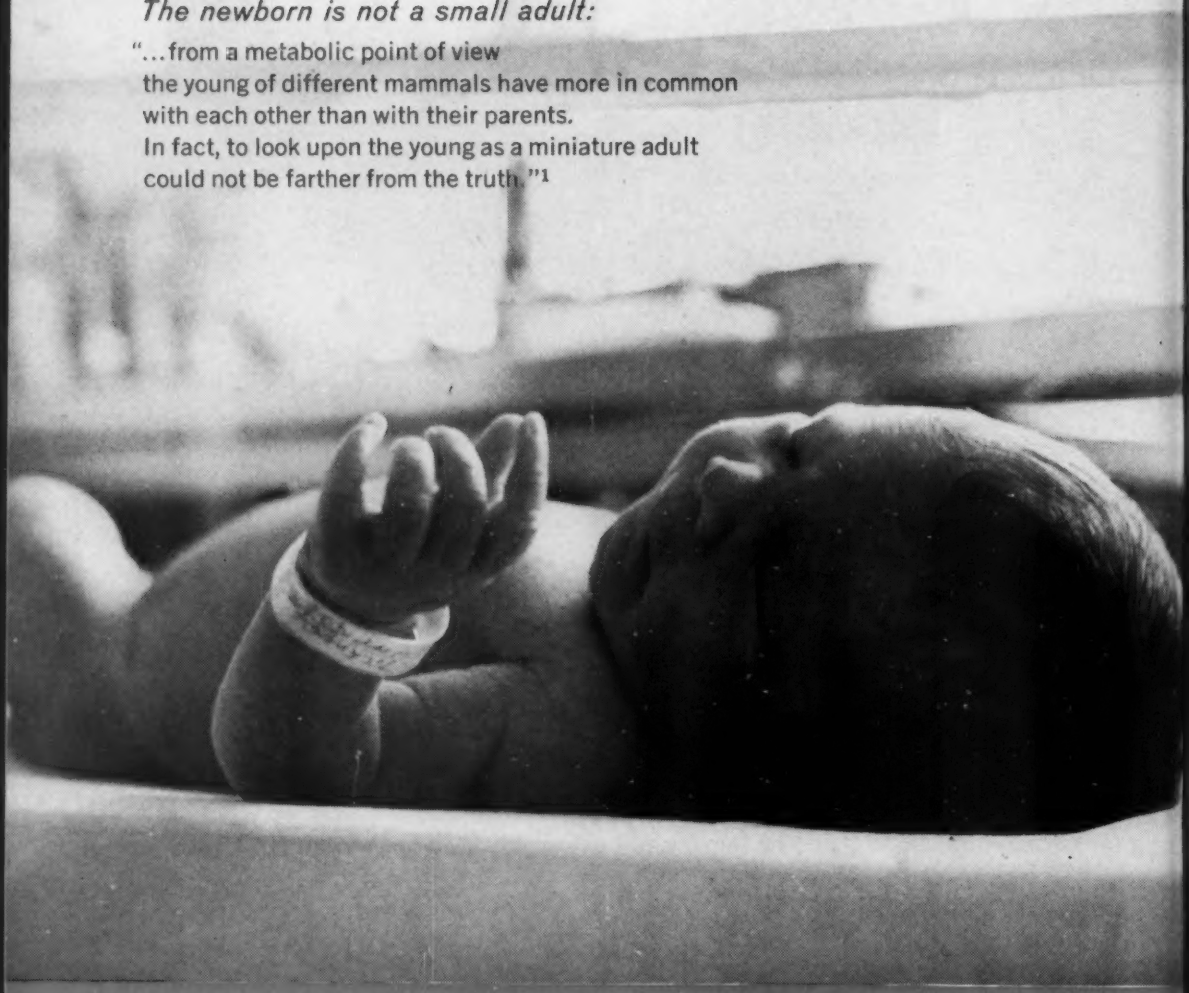
The newborn is not a small adult:

"...from a metabolic point of view
the young of different mammals have more in common
with each other than with their parents.
In fact, to look upon the young as a miniature adult
could not be farther from the truth."[1]



Physiologically and biochemically, the infant is a unique creature with very special characteristics. Just after birth and during the first week of life, adjustments to living apart from the maternal bloodstream result in blood chemistry patterns very different from those which are normal for adults.

Because of these differences, standards suitable for use in adult blood chemistry studies afford less assurance in determinations on the newborn, including the sometimes critically important bilirubin level.

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1. Lathe, G. H. (Sass-Kortsak, Andrew, ed.) in **Kernicterus;** report based on symposium held at IX International Congress of Paediatrics, Montreal, July, 1959. University of Toronto Press, Toronto, 1961.

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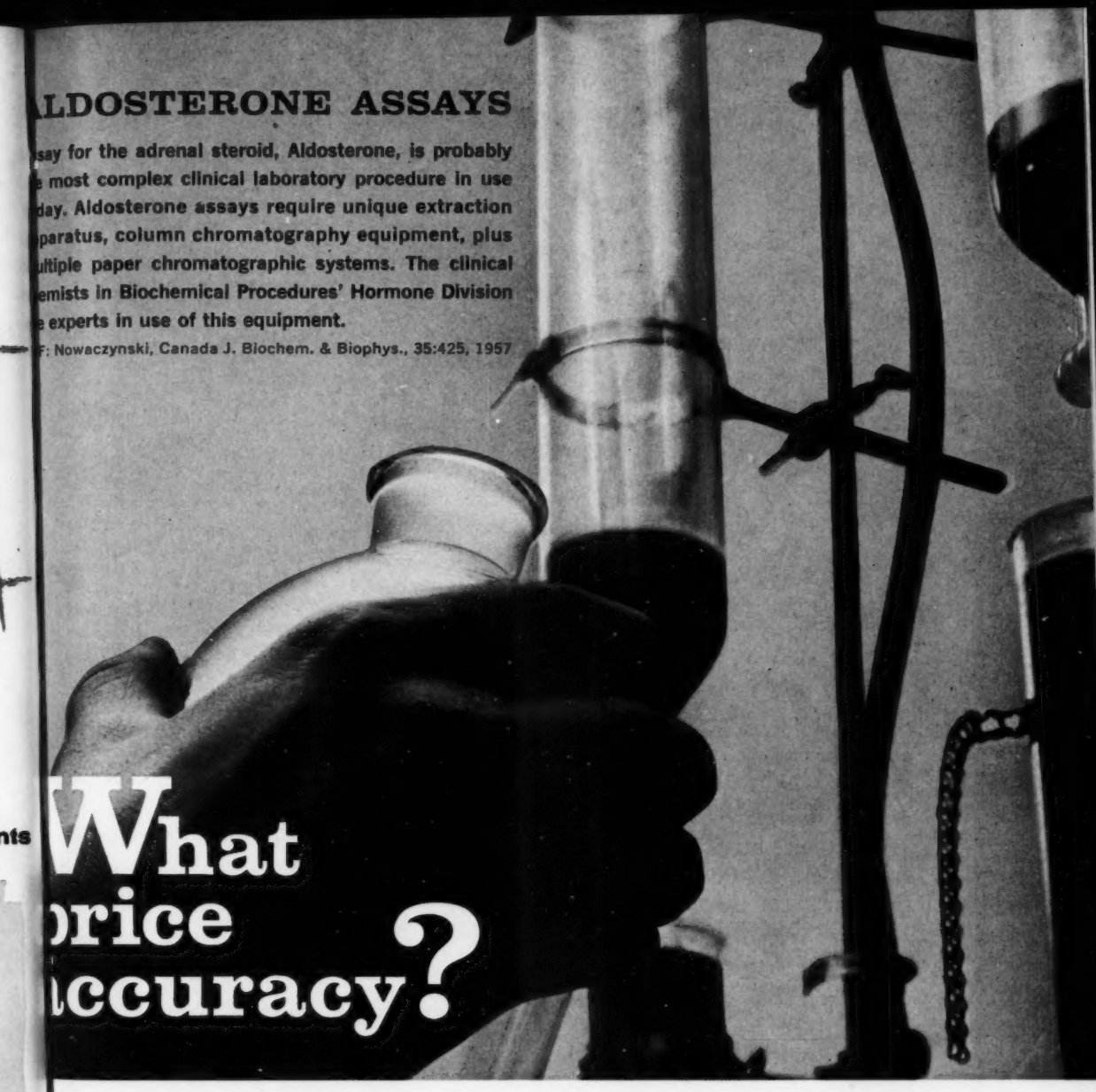

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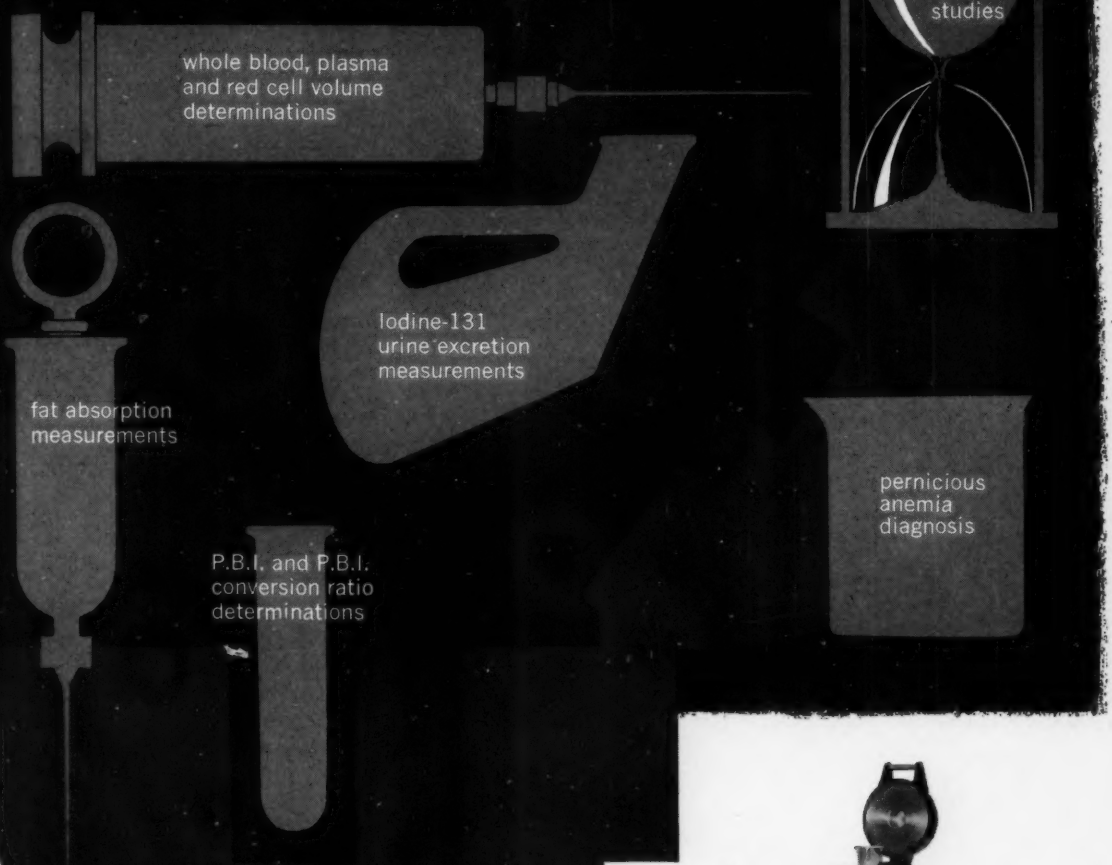
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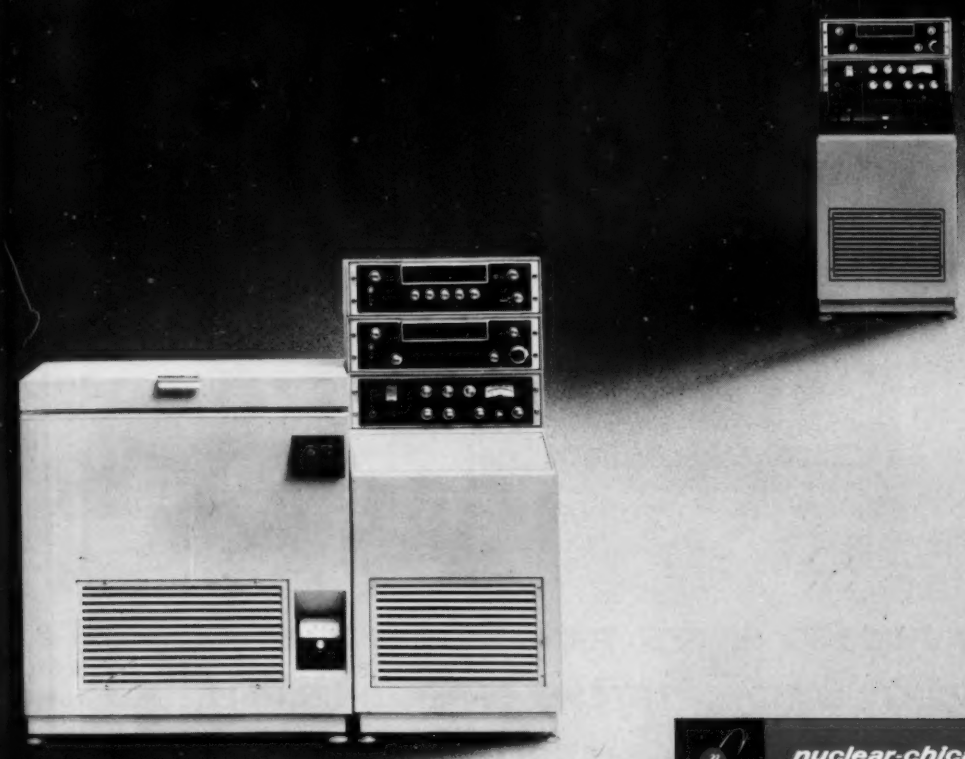

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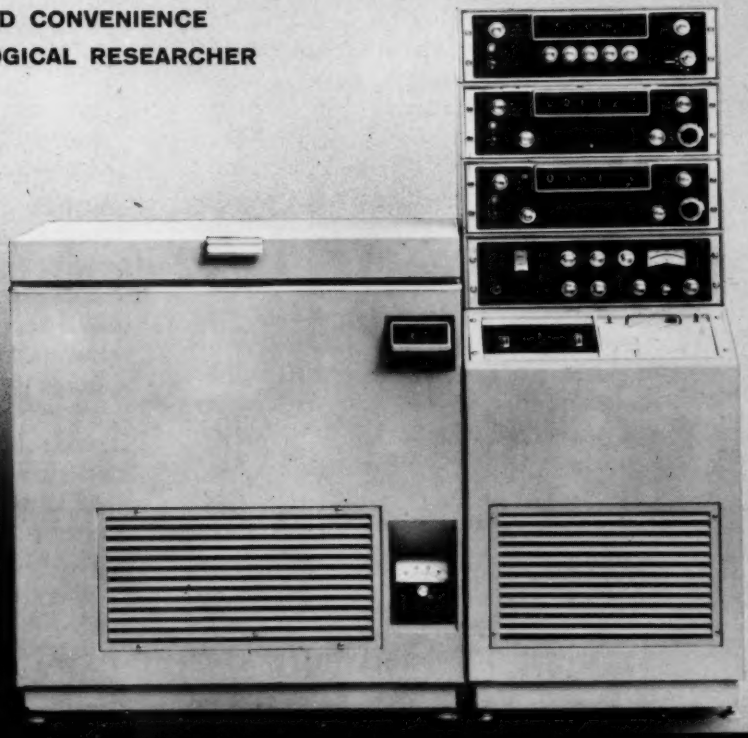
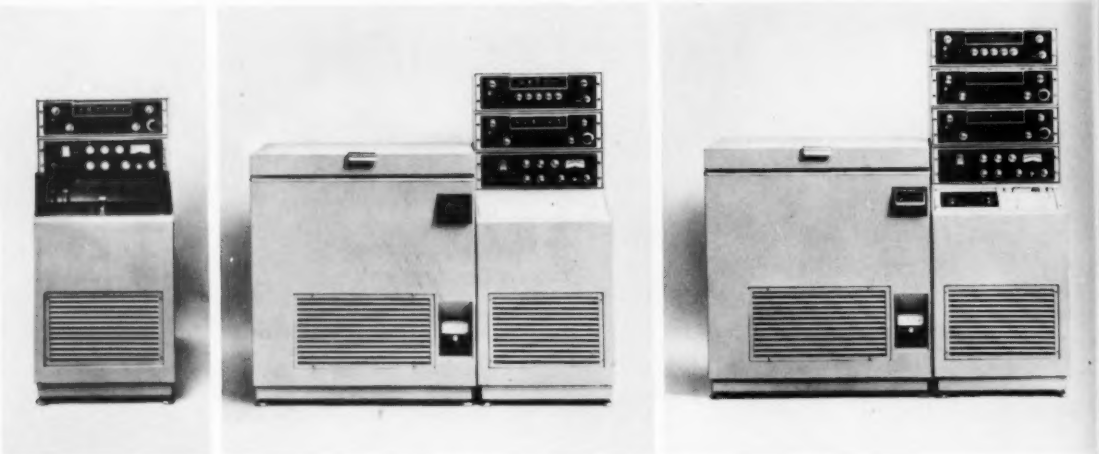


MODEL
701

MODEL
702

MODEL
703



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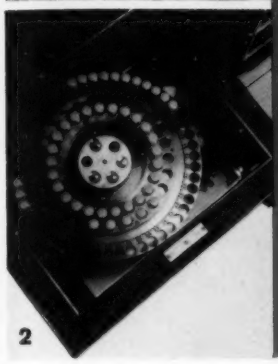
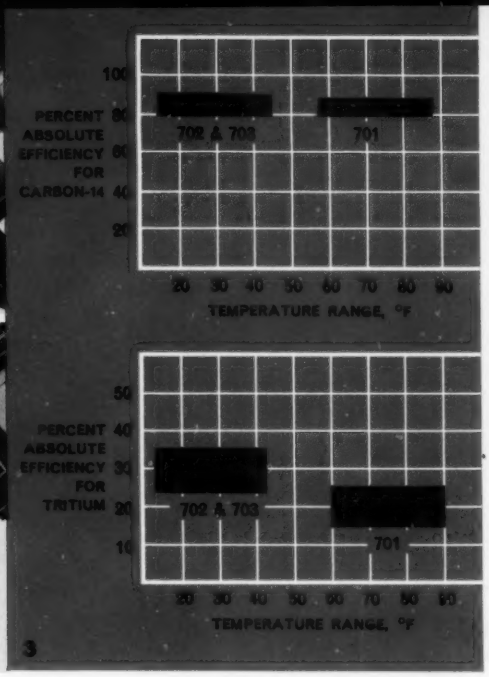
The Series 700—compatible with your present standard scaling instruments—easily accommodates to growth of research programs and equipment needs. The interchangeability of the unique dual detector, elevator assembly, solid-state electronics, and cabinetry permits the small laboratory to advance from one system to another as the budget grows—to go from *manual* operation at room temperatures with the 701, to manual *temperature-controlled* operation with the 702, and on to *automatic* temperature-controlled operation with the 703. Larger laboratories, requiring utmost precision for large numbers of samples, can obtain the 703 system with sophisticated, automatic readout — provided by Nuclear-Chicago's new Logic Series scalers, timer, and printer.

In all three systems, independent two-channel selection permits overlapping or separated channel counting. True double pulse resolution of less than one microsecond allows high speed counting with extremely small resolution losses. New, high gain photomultiplier tubes permit use of low electronic gain, providing vastly improved overload recovery characteristics while eliminating system "paralysis." Now, isotopes of widely differing energies and samples with count-rates varying from 500 cpm to 1,000,000 cpm can be counted alternately without gain shifts or memory effects causing loss of reproducibility.

MODEL 701 Liquid Scintillation System is chosen for economical, room temperature counting of carbon-14 and tritium. It is a modestly priced, high efficiency basic system with components common to all 700 Series models. The compact console houses a motor-driven elevator to position the sample in an optical cell; a dual-detector assembly; stabilized high voltage supply; fast coincidence circuitry; and a high speed, dual channel pulse-height analyzer. The detector assembly consists of high efficiency dual photomultiplier tubes shielded from external radiation and ambient temperature changes by steel and lead. A precision, automatically operated shutter eliminates light exposure to the photomultiplier tubes and permits use of the 701





1) Sample being placed on elevator of detector assembly in 702 controlled temperature chamber. Ample space is provided in cooling chamber for storage of samples.

2) Automatic sample changer in the 703 cooling chamber. Sample bottles are located at the edge of the circular table. Removable racks for additional samples and scintillation standards are provided.

3) Charts of 700 Series efficiency ranges obtained in integral counting. Sample used was labeled toluene with PPO and POPOP. For carbon-14 the 702 and 703 yield efficiencies of 80-90%, while the 701 provides an efficiency range of 80-85%. For tritium the 702 and 703 provide an efficiency range of 25-35%, while the 701 efficiency is 15-25%.

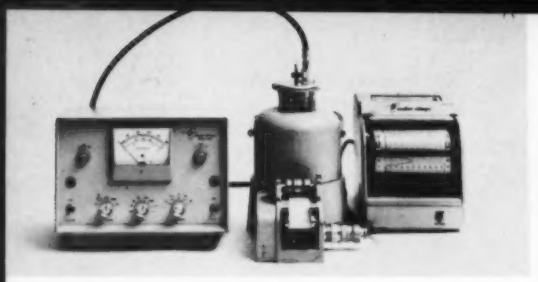
in ordinary room lighting. Output signals from the solid state circuitry are shaped pulses which can feed any standard scaling instrument. Nuclear-Chicago's Model 8250 Logic scaler is shown here mounted atop the 701 console.

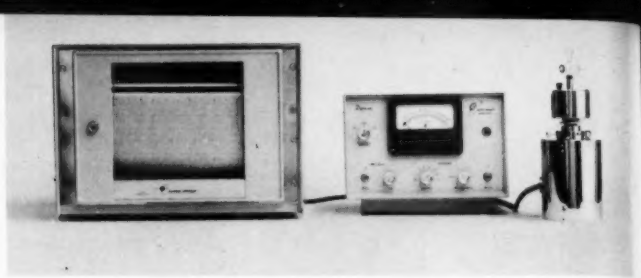
MODEL 702 has the dual-detector assembly mounted in a custom designed cooling chamber which permits highest efficiency for manual counting of C-14 and H-3 samples. The cooling chamber temperature is variable from +10° to +50°F and is stable with ±1°F. A pushbutton control on the front of the cooling chamber operates the motor-driven elevator which positions the sample in the dual detector assembly. Logic 8250 scaler and Logic 8600 timer are shown.

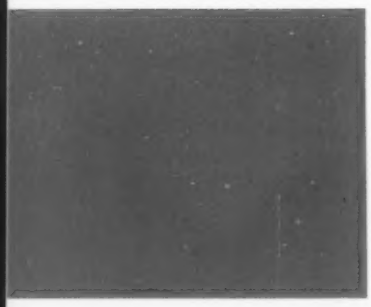
MODEL 703 is the most advanced counting system of its kind, providing both automatic sample changing and temperature controlled operation. Its sophisticated options of data presentation and versatile modes of automatic operation suggest it as the system of choice for laboratories counting many samples. A two-digit display on the cooling chamber shows the number of the sample which is in the counting position. The changer accepts up to 50 samples at a time, and an additional removable rack holds 50 more samples for temperature adaptation prior to measurement. Two 8250 scalers, 8600 timer, and 8401 digital recorder are shown.

Model 701

Model 703







C

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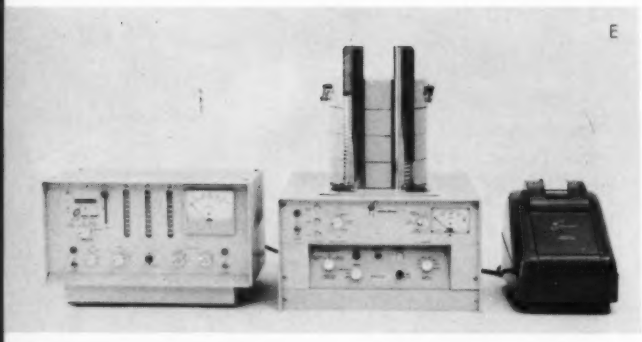
The systems presented here demonstrate the scope and completeness of Nuclear-Chicago's products for the biological researcher. They are—instrument by instrument, and system by system—capable of efficient, precise performance with unusual reliability. They are representative of the more than 250 instruments, counting systems, radionuclides and accessories presented in detail in Nuclear-Chicago's new, 96-page general catalog. Please send for your copy — you will find unequalled variety and versatility in the complete Nuclear-Chicago line.

A. C100B Actigraph II® automatically scans strip radiochromatograms.

B. 6000 Dynacon®Electrometer measures beta radiation in solid, liquid, or gaseous samples.

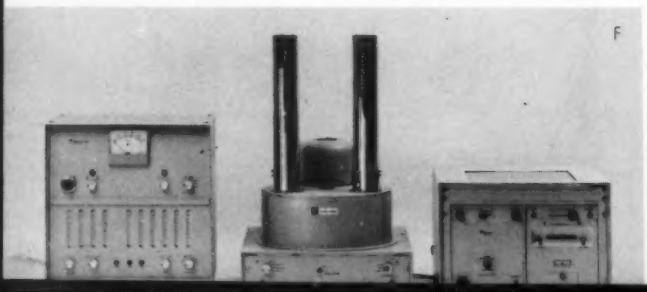


E

C. C120 Automatic Sample Changer accepts up to 49 gamma-emitting samples in test tubes. Scintillation well detector is mounted in console.

D. C110B Automatic Sample Changer accepts up to 35 solid beta samples 1 in. or 1¼ in. diameter.

E. C115 Automatic Beta Sample Changer is supplied with guarded gas-flow detector with background of only 2 cpm.



F

F. C210 Automatic Sample Changer accepts 50 or more beta samples up to 2 in. diameter.

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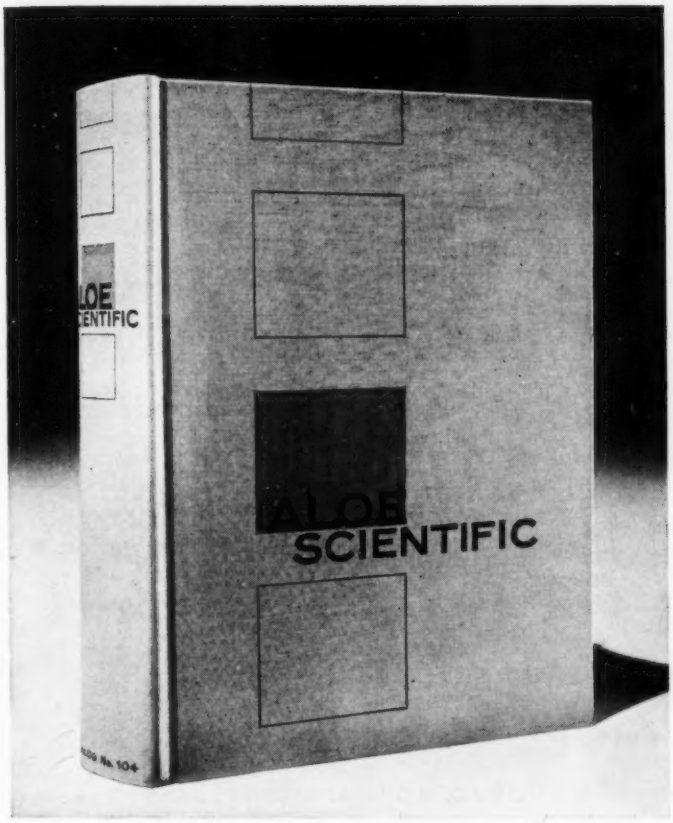

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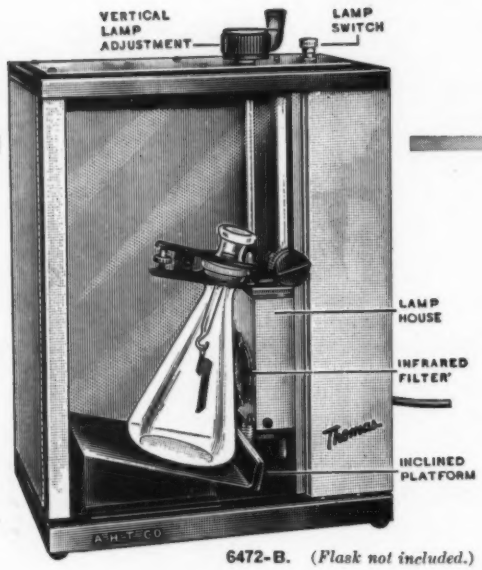
for oxygen flask combustions



6472-B. (*Flask not included.*)

- *Utilizes focused infrared beam for ignition*
- *Flask shielded completely during ignition and combustion*
- *Takes flasks 500 to 2000 ml capacity*

OXYGEN FLASK SAFETY IGNITER,* Thomas-Ogg. For combustion of organic materials in a closed oxygen flask completely shielded within a safety cabinet. Utilizes a focused infrared beam from a built-in 150-watt lamp and special black paper sample wrappers for rapid ignition in an oxygen-charged clamp-closed flask. Vertical alignment of the sample in the beam, and push-button firing are done from outside the cabinet after door is fastened.

Cabinet is of metal, 12¼ × 8 × 16 inches high overall, with transparent acrylic plastic door with full-length hinge, baffled vents and spring-loaded latch. Lamp is mounted in housing with screw crank elevating device; fixed focusing reflector is sealed within lamp envelope. Inclined platform tilts flask to bring sample close to lamp.

Designed for use with Thomas-Ogg combustion Flask, but accommodates any flask up to 2000 ml in which stopper can be clamped securely.

6472-B. Safety Igniter, Thomas-Ogg, as described, with flask platform, infrared filter, 100 black paper sample wrappers, extra lamp bulb, 3-wire cord, 3-prong plug with adapter and directions for use, but without combustion flask. For 115 volts..... **165.00**

OXYGEN COMBUSTION FLASKS,* Thomas-Ogg, of borosilicate glass, conical, designed especially for use in Ogg Safety Igniter but can also be used separately. Mouth is formed by socket of spherical joint 35/25, and stopper fabricated from matching ball member. Stopper has extension with hook for suspending the detachable sample carrier. Carrier is of perforated platinum sheet 1¾ inches long × ⅜-inch wide. Stopper is held securely during combustion by Thomas Pinch Clamp and can be tilted to permit easy release of vacuum following combustion.

6471-P10. Oxygen Flask, Thomas-Ogg, as described, 500 ml capacity, complete with stopper, clamp and platinum sample carrier, but without sample wrappers..... **41.70**
Each, in lots of 12 or more..... **39.65**

6471-P15. Ditto, but 1000 ml capacity.. **42.50**
Each, in lots of 12 or more..... **40.40**

NOTE—Two other types of Oxygen Combustion Flasks for infrared ignition have been developed at Cornell University by Donald J. Lisk and W. H. Gutenmann. *Information upon request.*

*Patent applied for. Based on developments by Clyde L. Ogg and associates, East. Util. Res. & Dev. Div., A.R.S., U.S.D.A., Phila., Pa.

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Clinical and experimental

Siderosis in the Bantu: The clinical incidence of hemochromatosis in diabetic subjects

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In a previous necropsy study on adult Bantu it was shown that when moderate or marked hepatic siderosis was present together with portal cirrhosis the findings elsewhere in the body were virtually indistinguishable from those in idiopathic hemochromatosis. Pancreatic deposits were particularly striking and diabetes was present in about 20 per cent of cases. In view of these findings, a random group of 100 adult Bantu diabetic subjects were investigated to discover what proportion were suffering from hemochromatosis. Plasma iron levels were estimated in all subjects and liver biopsies were then carried out on the majority of those in whom the plasma iron was high. Seven showed the changes of hemochromatosis. The clinical presentation in these subjects was different from that usually found in adult Bantu diabetic persons.

Although siderosis is common in Bantu adults, the iron deposits are usually confined to the liver and reticuloendothelial system.[1] The pathologic picture, therefore, is different from that found in idiopathic hemochromatosis, since widespread epithelial deposits are a striking feature in this disease.[2] On the other hand, there is some evidence that a small proportion of Bantu

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Table I. Liver and gastric biopsy findings in Bantu diabetics with high plasma iron leve

Diagnosis	*Case No.*	*Sex*	*Age*	*Plasma iron (μg/100 ml.)*	*Total iron-bindi capacity (μg/100 ml.)*	*Liver*
Hepatic siderosis with portal cirrhosis	1	F	60	171	250	++
	2	F	50	237	316	++
	3	F	67	198	237	++
	4	M	50	312	330	+
	5	M	55	180	216	++
	6	M	35	174	183	+
	7	M	56	246	300	++
Hepatic siderosis without cirrhosis	8	M	40	217	301	++
	9	M	42	196	226	++
	10	M	40	240	240	++
	11	F	40	240	240	++
Incomplete cases	12	M	47	165	235	
	13	M	50	222	248	
	14	M	45	232	243	

+ = Mild, − = Absent, ++ = Moderate, and +++ = Severe.

subjects do eventually develop pathologic changes similar to those found in idiopathic hemochromatosis. In one study the autopsy records of 20 known diabetic subjects were reviewed and in 4 the histologic and chemical findings were virtually indistinguishable from those in the idiopathic disease.[3] Since portal cirrhosis was a feature in each of these cases a further prospective investigation was carried out in which detailed chemical and histologic examinations were performed on every case of portal cirrhosis at autopsy in a Bantu hospital over an 18 month period.[4] The majority showed heavy hepatic siderosis and significant deposits were found in a number of epithelial tissues, with the highest concentrations in the pancreas. In addition, it was noteworthy that 22 per cent of the subjects were diabetic and that in each of them the chemical concentrations of iron in the pancreas were within the range of those found in idiopathic hemochromatosis.

These various observations strongly suggest that oral iron overload in the Bantu can eventually be associated with diabetes which is secondary to deposits in the pancreas. Since no diligent attempt has yet been made to diagnose such cases clinically a study was undertaken in which various tests were carried out on an unselected group of diabetic Bantu hospital patients. The plasma iron was estimated on all subjects and liver biopsies were performed on those in which it was raised. The presence of moderate or marked siderosis together with portal cirrhosis was taken as indicative of hemochromatosis, since in the previous pathologic study[4] significant pancreatic deposits invariably were present under such circumstances. In addition, gastric biopsies were carried out when possible.

Liver biopsy					*Gastric biopsy: Iron distribution*	
Iron distribution			*Portal cirrhosis*	*Portal fibrosis*		
Liver cells	*Kupffer cells*	*Portal tracts*			*Epithelium*	*Interstitium*
+++	+++	+	Present		–	–
+++	+++	+++	Present		+	+
+++	+++	+++	Present		–	+
++	++	++	Present		–	–
+++	+++	+++	Present		+	–
++	++	++	Present		+	–
+++	+++	+++	Present		+	?
+++	+++	+	–	++	–	+
+++	+++	+++	–	++	–	–
+++	+++	++	–	–		
+++	++	+	–	–		
					–	–
					–	–

Materials and methods

Clinical material. One hundred unselected Bantu diabetic subjects living in the urban area of Johannesburg were studied. Most of these were over 40 years of age. The majority were outpatients attending the Baragwanath Hospital Diabetic Clinic; the remainder were hospital patients. The diagnosis of diabetes mellitus was confirmed in all cases by blood sugar estimations.

Methods. The plasma iron level was estimated on all subjects by a modification of the method of Bothwell and Mallett,[5] in which sulfonated bathophenanthroline was used as the color reagent and the unsaturated iron-binding capacity was measured by a method of Bothwell and co-workers.[6] Liver biopsies were carried out in the majority of those with plasma iron levels above 150 μg per 100 ml. and gastric biopsies also were done in some. The specimens obtained were fixed in 10 per cent formol saline and sections were stained routinely with hematoxylin and eosin and, with Perl's method, for iron.

Results

A. Plasma iron levels. In 86 (29 men and 57 women) of the 100 diabetics the plasma iron levels were not raised. The mean value in men was 82 μg per 100 ml. (range: 34 to 126) and in women was 83 μg per 100 ml. (range: 41 to 139). The total iron-binding capacity varied from 192 to 475 μg per 100 ml. (mean: 291) in men; 168 to 458 μg per 100 ml. (mean: 306) in women.

Ten men and 4 women had plasma iron levels above 150 μg per 100 ml. (Table I). In each the finding was confirmed on a repeat estimation. The mean plasma iron level in this group was 216 μg per 100 ml. (range: 165 to 312) and the mean total iron-binding capacity was 255 μg per 100 ml. (range: 183 to 330).

B. Liver and gastric biopsy findings (Table I). Liver biopsies were carried out on 11 subjects with raised plasma iron levels. In 4 men and 3 women the liver biopsy showed moderate to marked siderosis, with iron present in parenchymal cells, Kupffer cells, and portal tracts. In addition, portal cirrhosis was present, the diagnosis being based on the presence of small, regular regenerating nodules surrounded by thin fibrous tracts and on the absence of collapse and gross architectural distortion. In 4 of the 7 patients gastric biopsy revealed epithelial deposits of iron. The absence of such deposits in the remaining 3 cases was not altogether surprising, since they were scanty or absent in about 50 per cent of a previous pathologic series of Bantu hemochromatotics.[4] All 7 cases were regarded as having fully developed hemochromatosis, and this was confirmed both histologically and chemically in 2 who subsequently died. The concentrations of pancreatic iron in these 2 subjects were found at autopsy to be 1.2 and 1.1 per cent dry weight, respectively.

In 3 men and 1 woman marked hepatic siderosis was present without cirrhosis. In 2 patients gastric biopsies were carried out but no epithelial deposits were found. It was felt that the pattern of iron distribution in these cases was that found in the majority of Bantu adults[1] and that it was extremely unlikely that epithelial deposits were present in the pancreas.

In the remaining 3 cases liver biopsy material was not obtained, either because the attempt at biopsy failed or because it was not possible to trace the patients. In 2 of these cases gastric biopsies were done and in both the findings were normal.

In order to test the validity of the screening procedure liver biopsies were carried out on 10 randomly selected cases from the control group with normal plasma iron levels. In 6 the findings were entirely normal, while the remaining 4 showed minimal or mild deposits of iron. In the 5 cases in which gastric biopsy was done the mucosa was found to be normal.

C. Clinical features of Bantu subjects with hemochromatosis. The clinical features in the 7 cases diagnosed on laboratory criteria during the study were analyzed (Table II, Cases 1 through 7). In addition, another 4 hospital patients who were not part of the random series were included also (Table II, Cases 8 through 11). In each of these 4 cases the plasma iron level was found to be high. Liver and gastric biopsies were then done in 3 of these cases. Marked hepatic siderosis together with cirrhosis was present in all of them and the epithelium of the stomach contained iron deposits in 2. The diagnosis of hemochromatosis was confirmed in the last case at autopsy, the concentration of iron in the pancreas being 0.7 per cent dry weight.

The findings in the 11 subjects were compared with those of 100 diabetics with normal plasma iron levels who were drawn at random from the Baragwanath Hospital Diabetic Clinic. The group was confined to patients over the age of 40 years since all but one of the subjects with hemochromatosis were over this age, as are three-quarters of all Bantu diabetics seen in hospital practice.

SEX. Of the 11 subjects with hemochromatosis 7 were men; there was a female-to-male ratio of 1.8 to 1.0 in the control group.

WEIGHT. Nine of the subjects with hemochromatosis were thin or wasted, 1 was of normal weight, and only 1 could be described as obese. In contrast about two-thirds of the control group were overweight.

CONSUMPTION OF ILLICITLY BREWED ALCOHOL. All subjects with hemochromatosis gave a history of consuming illicitly brewed alcoholic drinks. In the control group only 22 per cent admitted to taking such concoctions.

LIVER DISEASE. In every subject with hemochromatosis the liver was enlarged to palpation, often considerably so. The enlarged liver was usually firm in consistency. In the control group hepatomegaly was detected in only 10 per cent of cases. Evidence of liver failure or portal hypertension was present in 7 of the 11 subjects with hemochromatosis and was not seen in the control group.

PORPHYRIA. The urine was examined spectroscopically for porphyrins in 9 of the hemochromatotic subjects and excessive amounts were found in 3. Two of these cases showed the facial hyperpigmentation which is often present in Bantu cases of porphyria cutanea tarda.[7] Apart from these, the other subjects with hemochromatosis were not excessively pigmented. In the control group an excess of porphyrins in the urine was found in only 2 per cent of cases.

TUBERCULOSIS. Four of the subjects with hemochromatosis were found to have pulmonary tuberculosis as compared with an incidence of 9 per cent in the control group.

PROGNOSIS. The prognosis of patients with hemochromatosis was much more serious than that of subjects in the control group and 4 of them died within a year of the onset of symptoms. There were two major reasons for this. Liver failure and/or portal hypertension were present in 7 of the subjects with hemochromatosis and these complications were largely responsible for 3 of the 4 deaths in this series. Second, in about half the cases the diabetes could be described as severe. Other factors which probably contributed to the poor prognosis were alcoholism and the significant incidence of pulmonary tuberculosis. In contrast, the subjects in the control group were usually stable, ketosis was infrequent, and the diabetes could often be controlled by diet or by oral hypoglycemic agents.

Discussion

The results of a previous pathologic study indicated that when moderate or marked hepatic siderosis is found together with portal cirrhosis at autopsy significant epithelial deposits are always present in other organs, the highest concentrations being found in the pancreas.[4] On this basis 7 per cent of a random series of 100 diabetic Bantus attending hospital were diagnosed as having fully developed hemochromatosis. The incidence is an extremely high one when compared with the 0.16 per cent incidence of the idiopathic disease in an American series of 30,000 diabetic subjects.[8] It should, however, be emphasized that the present figures refer to a hospital population only.

Although the findings in Bantu diabetics with hemochromatosis resemble those found in idiopathic hemochromatosis there are certain notable differences. The cirrhosis in Bantu subjects tended to be rapidly progressive and was the principle cause of death, while in idiopathic hemochromatosis

Table II. Clinical features of 11 Bantu diabetics with hemochromatosis

Case No.	*Sex*	*Age*	*Illicit alcohol intake*	*Mode of presentation*	*Habitus*	*Signs of liver disease (finger)*	*Porphyria*	*Tubercle*	*Daily insulin dose*	*Remarks*
1	F	60	Moderate	Diabetic coma	Obese	4f. hepatomegaly	+	–	40	Enlarged heart on x-ray; no cardiac symptoms
2	F	50	Heavy	Diarrhea; glycosuria on routine urinalysis	Thin	4f. hepatomegaly	–	–	50	Stopped taking insulin thrice, followed by ketosis twice and death the third time; no autopsy obtained; course 9 months
3	F	67	Heavy	Polydipsia and polyuria	Thin	3f. hepatomegaly and 1f. splenomegaly			50	
4	M	50	Moderate	Polydipsia and polyuria	Thin	2f. hepatomegaly	+	+	80	Peri-orbital pigmentation
5	M	55	Moderate	Polydipsia, polyuria, and swelling of feet	Thin	3f. hepatomegaly, 2f. splenomegaly, ascites, leg edema, and gynecomastia	–	–	40	Death largely due to liver failure with progressive ascites, edema, wasting, and jaundice; course 8 months, hemochromatosis confirmed at autopsy
6	M	35	Heavy	Swelling of abdomen; glycosuria on routine urinalysis	Thin	2f. hepatomegaly and ascites	+	+	96	Patchy facial pigmentation; death mainly due to liver failure with progressive ascites, wasting, and hematemesis; course 11 months, hemochromatosis with ruptured varices at autopsy

7	M	56	Moderate	Clonic movements left leg and arm; glycosuria on routine urinalysis	Normal	3f. hepatomegaly and 1f. splenomegaly	–	–	See under *Remarks*	No cause found for clonic movements; initially stabilized on 120 units insulin daily but requirements fell until control achieved with tolbutamide alone
8*	F	45	Heavy	Cough and weight loss with pulmonary tubercle; glycosuria on routine urinalysis	Thin	4f. hepatomegaly and 1f. splenomegaly	–	+	40	
9*	M	49	Heavy	Weakness, headache, and polyuria	Thin	3f. hepatomegaly		+	80	
10*	M	70	Heavy	Abdominal pain	Thin	5f. hepatomegaly	–	–	20	First presented in 1955; jaundiced in 1957; diabetes discovered in 1960; one hypoglycemic episode while on 20 units insulin daily
11*	M	42	Moderate	Swelling of abdomen; glycosuria on routine urinalysis	Thin	4f. hepatomegaly, ascites, and edema	–	–	See under *Remarks*	Diabetes responded to soluble insulin but patient became comatose and died 4 days after admission; hemochromatosis with ruptured varices at autopsy

*These 4 cases were not part of the randomly selected series.

it is relatively benign and normally runs a protracted course.[2] There are several possible reasons for the difference. In addition to their high iron content[9] the alcoholic concoctions consumed by the Bantu are adulterated with a variety of toxic substances. It is feasible that these unidentified substances may, together with alcohol and malnutrition, be responsible for potentiating the fibrogenic action of the hepatic iron deposits and thus hastening the downhill course. Unknown toxic substances also may be responsible for the porphyria found in a significant proportion of Bantu cases but not seen in idiopathic hemochromatosis. With the cirrhosis dominating the clinical picture it is not surprising that cardiac complications, which are so common in idiopathic hemochromatosis,[2] are not a feature in Bantu cases, since the heart is one of the last organs to accumulate iron deposits[4] and the cirrhosis tends to cause death before they have reached significant proportions. One final difference between the present findings and those in idiopathic hemochromatosis was the high incidence of tuberculosis. Although there are several factors which probably contribute to its development, the high exposure rate among the Bantu population may well be the most important.

We wish to thank Dr. I. Frack, Superintendent of Baragwanath Hospital, and the Director, South African Institute for Medical Research, for granting facilities for this work.

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Siderosis in the Bantu: The relationship between iron overload and cirrhosis

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The Bantu of Southern Africa consume a diet with a high iron content and the majority of adults at autopsy show varying degrees of siderosis. Since previous studies have demonstrated a marked association between severe hepatic siderosis and portal fibrosis, an investigation was carried out to ascertain the pattern and degree of iron overload in consecutive cases of portal cirrhosis coming to autopsy. Severe hepatic siderosis was present in 22 out of 24 cases. In addition, iron deposits were found in a number of epithelial tissues including the pancreas, thyroid, and adrenals and the iron concentrations were within the range found in idiopathic hemochromatosis. These findings were different from those in a control group with severe hepatic siderosis but no cirrhosis. Under such circumstances the iron was confined to the liver and the reticuloendothelial system.

In idiopathic hemochromatosis there is an alteration in the behavior of the gut mucosa and excessive quantities of iron are absorbed from the diet.[1] By the time clinical manifestations appear, widespread deposits are present in most of the body tissues and the concentrations are particularly high in the liver and pancreas. The clinical manifestations of the disease are related closely to the iron deposits, since portal cirrhosis invariably is present and diabetes is found in approximately 80 per cent of cases.[1] There is, however, some dispute as to the role which iron plays in the production of tissue damage and it has been argued

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that the pathologic changes eventually found in the condition could equally well be a direct result of the metabolic defect that leads to excessive iron absorption.[2] If iron is itself the primary factor it might be anticipated that changes similar to those found in idiopathic hemochromatosis would occur in other forms of oral iron overload. In this context the findings in adult Bantu have special significance, since the majority show varying degrees of siderosis at autopsy.[3-5] In this condition there is good evidence that the most important etiological factor is the high iron content of the Bantu diet.[6]

Although iron overload is very common in Bantu adults it is not usually of a severe degree and it has been calculated that the total content of the body rarely exceeds 10 Gm., much less than is present in idiopathic hemochromatosis.[7] In such cases the iron is usually confined to the liver and the reticuloendothelial system.[4] Since the liver bears the major brunt of the iron load most investigators have concentrated on the findings in this organ. In early studies no correlation was found between the degree of hepatic siderosis and portal fibrosis,[4] but more recently a marked association has been noted.[7-9] In one of these investigations the iron concentrations in liver specimens were also estimated and it was found that hepatic fibrosis or frank portal cirrhosis was present in all those with iron concentrations above 2 per cent dry weight.[7] Because of these various findings a prospective study was undertaken to find out what proportion of subjects showing portal cirrhosis at autopsy had heavy iron deposits in the liver. In addition a number of other organs were examined so that a comparison could be made with the findings in idiopathic hemochromatosis. At the same time 2 other groups were examined, those with advanced hepatic siderosis but no cirrhosis, and those with postnecrotic cirrhosis.

Materials and methods

Consecutive cases of cirrhosis coming to autopsy over a period of approximately 20 months were examined both histologically and biochemically for their iron content. During this time approximately 700 autopsies were carried out and, of these, 38 showed cirrhosis. The distinction between portal and postnecrotic cirrhosis was based on certain morbid anatomic and histologic features previously described.[8, 10] In addition, 20 subjects with obvious macroscopic evidence of marked hepatic siderosis but with no cirrhosis were selected at random.

Postmortem tissues were fixed in 10 per cent formol mercury, embedded in paraffin wax, and stained with hematoxylin and eosin. In addition, they were stained for iron, using Perls' method. The iron concentration in most of the tissues was estimated chemically as previously described.[7] Results were expressed as a percentage per 100 Gm. dry weight.

Results

Severe siderosis without cirrhosis. Twenty subjects were examined. The average age was 51 years (range: 35 to 70 years). The striking biochemical findings were the high concentrations of iron in the livers and spleen. The mean concentration was 2.21 per cent (range: 0.86 to 7.55 per cent) in the liver and 3.38 per cent (range: 0.29 to 11.1 per cent) in the spleen. The average concentrations in other organs were as follows: pancreas, 0.09 per cent (range: 0.3 to 0.24 per cent); adrenal, 0.11 per cent (range: 0.03 to 0.26 per cent); thyroid,

0.08 per cent (range: 0.03 to 0.33 per cent); heart, 0.05 per cent (range: 0.03 to 0.13 per cent); testis, 0.07 per cent (range: 0.04 to 0.15 per cent); and kidney, 0.11 per cent (range: 0.04 to 0.62 per cent). These average concentrations are within or just above the normal range.[11]

Histologically, the iron in the liver was present in large amounts in hepatic cells, Kupffer cells, and portal tracts. There was a moderately close correlation between the concentration of iron and increased portal fibrosis. In the 10 cases where the concentration was 2 per cent or higher, portal fibrosis was severe in 7, moderate in 2, and mild in 1. On the other hand, in those cases with levels lower than 2 per cent, one had moderate portal fibrosis, 4 had mild portal fibrosis, and the remaining 5 were normal. These differences did not appear to be on account of disparities in age since the average ages in the 2 groups were 50 and 51 years, respectively. Apart from the liver and spleen, iron deposits were not striking in any other organs except the upper small bowel, where the iron was present mostly in the lamina propria. In other tissues it was confined totally to the reticuloendothelial cells and was never found in parenchymal cells.

The subjects in this group succumbed to a number of diseases. These included idiopathic heart failure (6 cases), miliary tuberculosis (3 cases), ruptured syphilitic aneurysms (2 cases), cerebral hemorrhage (2 cases), carcinoma of the stomach (1 case), peritonitis (1 case), rheumatic heart failure (1 case), pneumonia (1 case), pyelonephritis (1 case), acute pancreatitis (1 case), and unknown causes (1 case).

Postnecrotic cirrhosis. There were 14 cases in this group, an incidence of 2 per cent. The average age was 40 years (range: 18 to 60 years). There was a wide variation in the iron concentrations in liver (0.03 to 1.87 per cent) and in spleen (0.06 to 4.10 per cent). The average levels of 0.38 and 0.67 per cent, respectively, however, were much lower than are found in apparently normal Bantu in the same age groups.[7] Iron concentrations in other organs, with one exception, were within the normal range. This subject had the highest concentration in the liver (1.87 per cent) and the concentrations in other organs were as follows: spleen, 1.31 per cent; pancreas, 1.22 per cent; adrenal, 0.14 per cent; thyroid, 0.10 per cent; pituitary, 0.11 per cent; heart, 0.22 per cent; testis, 0.02 per cent; and kidney, 0.08 per cent.

In 13 of the 14 cases, iron, when present, was confined to the liver and reticuloendothelial system. In the last case, which was the one with the highest hepatic concentration, deposits were also present in parenchymal cells elsewhere in the body; those in the pancreas were particularly striking.

Seven subjects died from malignant hepatomas, 4 from ruptured varices, 2 from hepatic failure, and 1 from rheumatic heart failure.

Portal cirrhosis. There were 24 cases of portal cirrhosis, an incidence of 3.4 per cent. Three of the cases were of the fatty or nutritional variety. Excessive hepatic deposits of iron were a feature in all except 2 cases, 1 of which was fatty (Table I). The livers of the remaining cases were macroscopically brown in color due to the iron deposits. The average iron concentration in the whole group was 2.31 per cent (range: 0.07 to 8.16 per cent). In the spleen the iron concentration was also very high, with a mean value of 2.63 per cent (range:

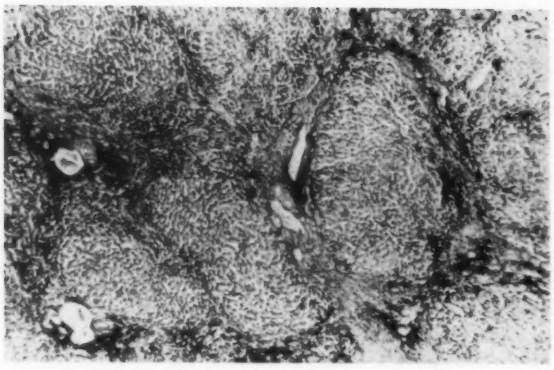

Fig. 1. Section of the liver showing portal cirrhosis and heavy hemosiderin deposits in hepatic cells, Kupffer cells, and portal tracts. (Perls' stain. Original magnification × 30.)

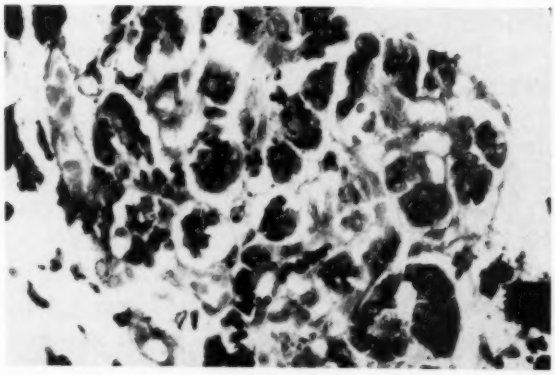

Fig. 2. Section of the pancreas showing hemosiderin deposits in the acinar-lining cells. (Perls' stain. Original magnification × 600.)

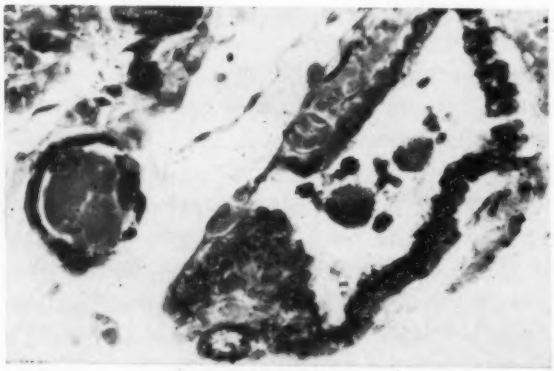

Fig. 3. Section of the thyroid showing hemosiderin in the epithelial cells lining the follicles. (Perls' stain. Original magnification × 600).

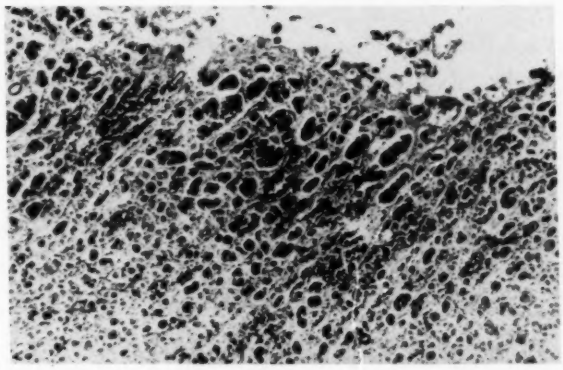

Fig. 4. Section of the adrenal gland showing heavy hemosiderin deposits which are mostly in the secreting cells of the zona glomerulosa. (Perls' stain. Original magnification × 120.)

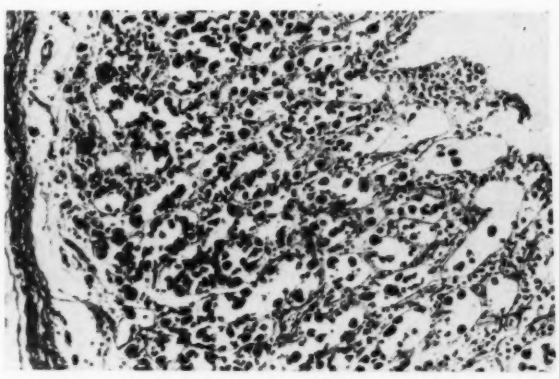

Fig. 5. Section of the stomach mucosa showing large deposits of hemosiderin in the epithelial cells particularly in the deeper layers. (Perls' stain. Original magnification. × 200.)

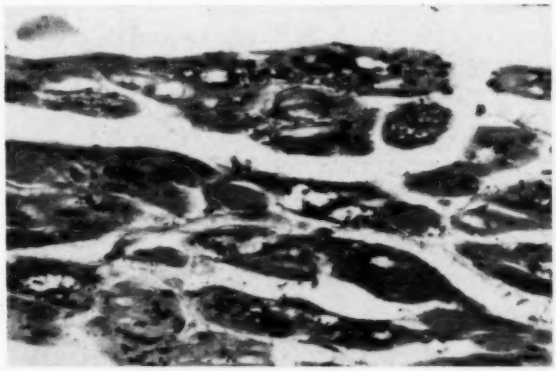

Fig. 6. Section of heart muscle showing heavy accumulations of hemosiderin in the myofibrils. (Perls' stain. Original magnification × 600.)

Table I. Iron concentrations (per cent dry weight) in organs of subjects with portal cirrhosis

Case No.	*Sex*	*Age*	*Liver*	*Spleen*	*Pancreas*	*Adrenal*	*Thyroid*	*Pituitary*	*Heart*	*Testis*	*Kidney*	*Cause of death*
1	M	75	8.16	3.65	2.01	1.20	0.55	—	0.10	0.26	0.18	Hepatic failure
2	M	37	6.84	7.36	0.69	0.14	—	—	0.08	0.21	0.14	Hepatic failure, tuberculosis
3	F	61	5.25	3.02	0.86	—	—	—	0.14	—	0.25	Hepatic failure
4	F	80	4.63	5.64	0.45	0.62	0.12	0.11	0.07	—	0.12	Cerebral hemorrhage
5	M	29	3.15	4.14	0.51	0.20	0.11	—	0.06	0.83	0.08	Malignant hepatoma
6	M	31	3.01	5.77	1.17	0.30	0.44	—	0.27	0.48	0.12	Hemothorax (? cause)
7	M	68	2.71	2.70	0.27	0.12	—	0.09	0.23	0.05	0.07	Hepatic failure
8	M	35	2.24	2.94	1.10	0.61	0.82	0.22	0.17	0.11	0.14	Ruptured varices (diabetes)
9	M	39	2.13	0.31	2.98	0.24	0.70	0.34	0.31	—	0.07	Ruptured varices
10	M	74	2.12	1.41	0.16	0.29	0.10	0.14	0.05	0.05	0.06	Pyelonephritis, renal failure
11	M	42	2.12	1.30	0.73	0.38	0.17	0.25	0.13	0.13	0.15	Ruptured varices (diabetes)
12	F	60	2.00	1.54	0.70	0.23	0.94	0.18	0.20	—	0.13	Hepatic failure
13	M	51	1.90	4.20	—	0.36	0.26	0.20	0.07	—	0.41	Ruptured varices
14	F	57	1.50	1.52	1.06	0.40	0.84	0.33	0.08	—	0.10	Ruptured varices
15	F	29	1.37	4.12	—	—	0.03	—	0.03	—	0.05	Hepatic failure
16	F	44	1.30	0.90	2.20	—	0.60	—	0.80	—	—	Diabetic coma and hepatic failure
17	M	48	0.93	1.55	0.57	—	0.20	—	0.09	—	0.16	Meningitis, pyelonephritis and hepatic failure
18	M	41	0.84	1.15	1.71	0.40	0.08	—	0.48	0.01	0.11	Hepatic failure and bronchopneumonia
19	F	80	0.84	0.83	0.41	0.09	0.45	0.16	0.10	—	0.09	Hepatic failure (diabetes)
20*	M	32	0.84	6.13	0.08	0.24	0.05	0.10	0.08	0.06	0.07	Hepatic failure
21	M	55	0.77	0.87	1.22	0.10	0.16	—	0.18	0.07	0.09	Hepatic failure (diabetes)
22	M	49	0.48	1.05	0.81	0.09	0.17	—	0.07	0.05	0.07	Hepatic failure
23*	F	40	0.12	0.59	0.04	0.04	0.04	—	0.03	—	0.04	Hepatic failure and ruptured varices
24*	F	50	0.07	0.49	—	—	0.05	0.05	0.02	—	0.04	Hepatic failure and widespread tuberculosis
Average		51	2.31	2.63	0.94	0.32	0.33	0.18	0.16	0.20	0.12	

*Fatty liver.

0.31 to 7.36 per cent). In addition, significant deposits were present in a number of other organs. The average concentrations were as follows: pancreas, 0.94 per cent; thyroid, 0.33 per cent; adrenal, 0.32 per cent; testis, 0.20 per cent; pituitary, 0.18 per cent; heart, 0.16 per cent; and kidney, 0.12 per cent.

On histologic section the liver showed heavy deposits in hepatic cells, Kupffer cells, and portal tracts. A striking feature in this group was the presence of excessive iron in organs other than the liver and spleen. Deposits were particularly prominent in the pancreas, where they were found in both the acinae and islets of Langerhans. In the thyroid the deposits were almost exclusively in the follicular-lining cells, while in the adrenal the zona glomerulosa of the cortex was most affected. Hemosiderin granules were present in myocardial fibers in 15 cases and in 5 of these the deposits were moderate or heavy. Involvement of the epithelial cells of the stomach was noted in 16 cases but in 6 they were extremely scanty and irregular in distribution. Representative sections of various tissues from Case 6 (Table I) are shown in Figs. 1 through 6.

Discussion

The present study was undertaken to find out the pattern and degree of iron overload in the South African Bantu showing cirrhosis at autopsy. Since iron overload is so common in the Bantu, a control group of cases showing marked hepatic siderosis without cirrhosis was also examined. In this group the features were similar to those found by previous workers since the iron deposits were virtually confined to the liver and reticuloendothelial system.[4] Although varying degrees of portal fibrosis were present in all cases in which the hepatic concentration of iron was above 2 per cent dry weight, death did not seem to be attributable to the iron deposits since the subjects succumbed to a variety of unrelated causes.

In postnecrotic cirrhosis, variable deposits were found but the average concentration in the liver was a good deal lower than is found in the general Bantu population at comparable ages. It therefore appears that excessive deposits of iron play no part in the genesis of postnecrotic cirrhosis and when the two do occur together it is only because varying degrees of iron overload occur so commonly in the Bantu.

In cases with portal cirrhosis the pattern was completely different since iron overload was a feature in all except 2 persons. The mean concentration in the liver was approximately twice as great as occurs at comparable ages in the general population[7] and, in fact, was very similar to that obtained in the group selected solely because of marked hepatic siderosis. The second feature of note was the distribution of the iron. Not only was it present in liver and reticuloendothelial system but in addition was found in a number of epithelial tissues. The histologic findings were therefore virtually indistinguishable from those in idiopathic hemochromatosis and the only feature that served to distinguish the two conditions was the relatively high concentration of iron in the spleens of Bantu cases.

These results raise the question as to the role which iron deposits play in the production of portal cirrhosis. In both this study and a previous one[7] it was

demonstrated that the incidence of portal fibrosis increases as the concentration of iron in the liver rises. This suggests that there is a direct association between iron deposits and fibrosis. However, it is noteworthy that several of the cases of portal cirrhosis in the present series had concentrations of iron in the liver which were lower than those with marked siderosis but no cirrhosis. This difference was not explicable on age or sex differences. Although it is feasible that the iron concentration in a single aliquot of cirrhotic liver may not necessarily be a valid reflection of the over-all concentration, these findings raise the possibility that some other factor such as malnutrition or an extrinsic "toxin" may accelerate the development of cirrhosis in some subjects. It has been shown experimentally that cirrhosis develops rapidly in the liver overloaded with iron when the animal is exposed to toxic agents or to a deficient diet[12] and it is possible that a similar situation may exist in the Bantu. It should be noted that a large proportion of the iron ingested by the Bantu is derived from the containers used in the preparation of alcoholic drinks so that those with marked siderosis are usually heavy drinkers. It is therefore possible that the insult of alcoholism may contribute to the subsequent cirrhosis.

The second problem relates to the effect of portal cirrhosis on the distribution of iron within the body since it is apparent from the data that parenchymal deposits occur only in other organs when cirrhosis is present. This pattern of distribution does not seem to be related to the type of cirrhosis, as it was also found in 1 case of postnecrotic cirrhosis with severe hepatic siderosis. Although it is possible that the raised plasma iron levels and the increased saturation of circulating transferrin occurring under such circumstances may facilitate epithelial uptake, there are at present insufficient data to support this.

Whatever the explanation for the findings in the present study, there is no doubt that a proportion of Bantu adults develop pathologic findings which are virtually indistinguishable from those in idiopathic hemochromatosis. The similarity between the two conditions is apparent in the clinical manifestations also. Apart from symptoms referable to portal cirrhosis, 5 out of 22 patients were diabetic, an incidence of 22.7 per cent. This is 10 times greater than the 2. 2 per cent incidence which has been found previously in 700 nonhemochromatotic subjects of comparable age distribution coming to autopsy at the same hospital[13] and it is also several times greater than the reported incidence of diabetes in cirrhotic subjects without siderosis.[14] Although the incidence of diabetes is still much lower than that found in idiopathic hemochromatosis,[1] this is possibly because of the fact that the cirrhosis in the Bantu is more active and severe. Death, therefore, may supervene in the majority of cases before epithelial deposits have reached sufficient concentrations to give rise to pathologic effects.

We wish to thank Dr. I. Frack, Superintendent of Baragwanath Hospital, for access to case records, and the Director, South African Institute for Medical Research, for granting facilities.

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Comparison of in vitro and in vivo methods of erythrocyte tagging with Cr^{51}

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A valid erythrocyte survival curve may be obtained in the golden hamster by any of 4 techniques of chromation in vitro or in vivo. The method of choice appeared to be the intraperitoneal administration of $Na_2Cr^{51}O_4$.

The principal concentration of radioactivity 7 days or more after erythrocyte chromation is in the liver and spleen, indicating the important role of these organs in the destruction of erythrocytes.

As a label for the determination of erythrocyte survival, Cr^{51} has been widely employed.[1-9] The intravenous return of erythrocytes incubated with sodium chromate ($Na_2Cr^{51}O_4$) in vitro gives reproducible levels of specific circulating radioactivity for study of blood volumes and red cell longevity in man and large animals.[2] However, application of this procedure to the study of laboratory animals with very small blood volumes has, in some instances, been attended by considerable technical difficulty and for this reason a modified technique of chromium tagging in vivo has been devised.[6] The intraperitoneal injection of $Na_2Cr^{51}O_4$ has proved an efficient and practical means of labeling the erythrocytes in normal hamsters, producing Cr^{51} survival data very similar to those obtained by the intracardiac reinjection of erythrocytes labeled in vitro. Peak levels of red cell radioactivity were obtained 1 to 4 days after injection and Cr^{51} disappeared completely from the circulating plasma after 4 to 7 days. In the hope of reducing the time required for red cell chromation, while retaining the advantages of the intraperitoneal technique, $Na_2Cr^{51}O_4$ was administered

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to hamsters by the intracardiac route. The subsequent uptake of Cr^{51} by circulating red cells was measured and the distribution of this label throughout the body was determined at the time of death.

Materials and methods

Four groups of golden hamsters (*Mesocricètus auratus*) weighing 60 to 80 grams and fed Purina Chow and water ad libitum were used in this study. Erythrocyte chromation in vivo, by intraperitoneal injection of $Na_2Cr^{51}O_4$, and the procedure for calculating erythrocyte survival in the hamster have been reported earlier.[6] The technique of Ebaugh, Emerson, and Ross[2] was followed for the chromation of red cells in vitro.*

Group I. Chromation in vitro and intravenous reinjection of erythrocytes. One milliliter of whole blood was withdrawn from each of 6 hamsters by cardiac puncture, mixed with 0.4 ml. A-C-D diluent, and incubated at 37° C. for 1 hour with 30 μc of Cr^{51} ($Na_2Cr^{51}O_4$). The samples were shaken gently every 10 minutes during incubation. Following incubation, the blood was centrifuged at 1,500 r.p.m for 5 to 10 minutes, after which the plasma was withdrawn and replaced by normal saline. The labeled red cells were then returned intravenously via the femoral or jugular vein or by intracardiac injection. Blood samples (0.1 ml.) were obtained by cardiac puncture from each animal on day 1 and at 2 to 7 day intervals thereafter. Specific hemoglobin radioactivity was determined in each sample.

Group II. Chromation in vitro and intraperitoneal reinjection of erythrocytes. One milliliter of hamster blood was obtained from each of 7 hamsters, labeled as above, then reinjected intraperitoneally. Blood specimens were then obtained, processed, and tested as described for Group I.

Group III. Chromation in vivo and intracardiac injection of Cr^{51}. Thirty microcuries of Cr^{51} ($Na_2Cr^{51}O_4$) in 0.3 to 1.0 ml. sterile solution was injected by cardiac puncture into the circulating blood of 4 hamsters and erythrocyte survival determined as in Group I.

Group IV. Chromation in vivo and intraperitoneal injection of Cr^{51}. Thirty microcuries of Cr^{51} ($Na_2Cr^{51}O_4$) in 0.3 to 1.0 ml. sterile solution was injected intraperitoneally into 6 hamsters and erythrocyte survival measured as in Group I.

Blood samples for determination of red cell survival were separated into their plasma and cellular fractions by mechanical sedimentation and the Cr^{51} concentration determined in each portion.

Animals were sacrificed after 7 to 50 days and autopsies were performed. The specific radioactivity in representative samples of livers and spleens was then determined, using a technique previously described.[6] In 5 animals the peritoneal cavity was washed with normal saline at intervals and specific radioactivity of the total volume of washings was determined (Table I). The ability of the Cr^{51} in these washings to chromate erythrocytes in vitro was also determined in 4 of these animals. Perfusion of the liver with saline prior to tissue sampling resulted in no appreciable difference in hepatic radioactivity as compared to that in nonperfused livers.

Results

Cr^{51} counts varied from 200 to 20,000 per 0.1 ml. whole blood per minute with background values of 130 to 160 counts per minute.

Radioactivity was present in both plasma and erythrocytes for 4 to 7 days after Cr^{51} injection in Groups III and IV (chromation in vivo). Thereafter, appreciable radioactivity was limited to the erythrocyte portion. The peak level of radioactivity in labeled erythrocytes which had been reinjected intraperitoneally (Group II) occurred between 1 and 4 days. The 4th day was therefore chosen as a reference point for calculating the 100 per cent value of

*We are grateful for technical assistance rendered by Dr. Belton Burrows and the Department of Radioisotopes, Massachusetts Memorial Hospitals, Boston, Mass.

Table I. Organ distribution of radioactivity in normal hamsters with Cr^{51}-labeled red cells

Method of chromation	*Days following chromation*	*Organ*	*Organ weight (Gm.)*	*Counts per minute per Gm.*	*Counts per minute per organ*
Injection of intravenous Cr^{51}-labeled erythrocytes	7	Liver	5.00	20,000	100,000
		Spleen	0.15	424,000	63,000
		Kidney	0.55	1,900	1,050
		Bone		7,590	
Injection of intraperitoneal $Na_2Cr^{51}O_4$	5	Liver	4.00	41,000	164,000
		Spleen	0.10	95,400	9,540
		Kidney	0.35	44,000	15,485
		Bone		16,000	
		Lung	0.13	6,700	875
		Muscle		1,820	
		Peritoneal washings			15,065

Table II. Disappearance of circulating radioactivity in hamsters with Cr^{51}-labeled red cells

Group	*Labeling Methods*	*No. of animals*	*Cr^{51} half period (days) Mean ± 2 standard deviation*	
I	Intravenous injection of chromated red cells	6	14.5	5.0
II	Intraperitoneal injection of chromated red cells	7	11.0	3.6
III	Intravenous $Na_2Cr^{51}O_4$	4	14.0	6.2
IV	Intraperitoneal $Na_2Cr^{51}O_4$	7	16.5	7.8

radioactivity in Groups II, III, and IV. The 24 hour value was chosen as a reference point for the calculations in Group I. The washings from the peritoneal cavity contained significant radioactivity at least until 5 days after intraperitoneal injection, and capacity for tagging normal hamster erythrocytes with Cr^{51} could be demonstrated in the washings at 24 and 48 hours, but not at 96 hours.

The erythrocyte survival curves in normal hamsters are depicted in Fig. 1. The half period of red cell radioactivity ranged from 11 to 20 days in Groups I, III, and IV. In Group II (intraperitoneal red cells) the half period of red cell radioactivity ranged from 8 to 15 days. The mean chromium half times for all groups are expressed in Table II. The extinction values, determined by extrapolation, appeared to reflect comparable erythrocyte longevity in all groups. The uptake of Cr^{51} by circulating erythrocytes (10 to 30% of total

dose) using the same amount of Cr^{51} in each group (30 μc) was greatest in Group IV and least in Group II.

The ratio of significant radioactivity per gram of tissue of liver and spleen determined 7 days or more after chromation ranged from 0.1 to 0.54 c.p.m. per gram, with overlap in all 4 groups of animals. The average liver weight in these animals was 4.5 grams (3.0 to 6.5 grams) and the mean ratio of liver weight to animal weight was .046. The ratio of significant radioactivity per total organ (liver/spleen) varied widely from 1.6 to 96.0.

Significant radioactivity was also encountered in the kidneys and bone marrow of all animals tested. For the kidney, activity was highest in Groups III and IV (Table I).

Discussion

The erythrocyte survival curve in the golden hamster has been determined using four different methods. The best agreement in Cr^{51} half period has been obtained in Groups I, III, and IV. The intraperitoneal injection of red cells labeled with Cr^{51} (Group II) results in a somewhat shorter mean chromium half period, although the morphology of the curves is similar and survival values in all groups overlapped.

The maximum acceptable concentration of $Na_2Cr^{51}O_4$ compatible with normal red cell longevity for use in chromation in vitro has been estimated to be 30 μc per milliliter of whole blood.[7] Using this amount of $Na_2Cr^{51}O_4$ in vitro, difficulty was encountered in obtaining high specific activity of circulating erythrocytes, especially in Group II. Erythrocyte survival in this group could be followed for only 15 days, at which time, however, the biologic half period of Cr^{51} activity in these animals had elapsed. This technical factor nevertheless may account in part for apparent differences in length of erythrocyte survival.

The use of intraperitoneal $Na_2Cr^{51}O_4$ for determination of erythrocyte survival appears to be as reliable as any of the other methods and is associated with fewer technical problems. The delay in the development of peak erythrocyte radioactivity for as long as 4 days is probably related to the persistence

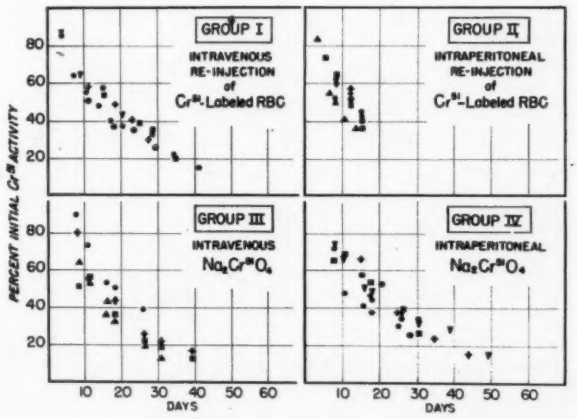

Fig. 1.

of hexavalent Cr^{51} in the peritoneal cavity. Another possible explanation might be that the radioactive label was attached in the marrow to immature erythrocytes which then entered the peripheral blood over a period of several days. The persistence of plasma radioactivity for 4 to 6 days is undoubtedly influenced by this sequestration of Cr^{51} and also by labeling of other blood elements.

The distribution of organ radioactivity as observed post mortem may indicate not only loci of erythrocyte destruction, but also foci of Cr^{51} binding to nonerythrocyte elements during the 4 to 7 day postinjection period. The predominance of specific radioactivity in the liver and spleen after 7 days indicates that these organs of the reticuloendothelial system are largely responsible for the destruction of red cells in the normal hamster.

The fact that Cr^{51} labeled red cells and $Na_2Cr^{51}O_4$ in solution cross the peritoneal barriers to give significant amounts of radioactivity in the circulating blood of hamsters is of interest. In preliminary exploitation of this observation, we have successfully employed the intraperitoneal injection of $Na_2Cr^{51}O_4$ in human subjects in this laboratory to determine erythrocyte survival.

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Observations on body water in oliguric patients

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Determinations of exchangeable body water in oliguric patients are few, and only rarely has tritium been used for this purpose. In this study, body water was determined during oliguria in 12 subjects. A test was made of the determination of body water by urea measurements during the course of hemodialysis using both tritium and urea. For the standard dilution method using tritium, the time course of equilibration in the bedridden, oliguric patient was shown to be identical to that commonly observed in ambulatory, healthy, normal persons; namely, equilibration occurs after 2 hours. The state of hydration in the oliguric patients was carefully maintained in the normal range, observing the precaution of giving replacement fluids with a deduction for endogenously produced water. Under these circumstances, too, exchangeable body water expressed as percentages of body weight accurately reflected the absolute changes, in those cases where sequential determinations were carried out. The use of urea to ascertain body water during hemodialysis gives results similar to those obtained by the conventional tritium method. However, this is shown to be a consequence of the fortuitous cancellation of 2 oppositely directed errors inherent in the urea technique. For this reason, the urea technique may be potentially unreliable.

In the conservative management of the oliguric patient, attention is focused primarily on fluid balance and the status of bodily hydration. In the absence of renal regulation, one would suspect the trend to be an increase with time in body water. This would occur because exogenous provision of replacement volumes generally fails to allow for additions to the total body water from endogenous sources[1, 2] and is, therefore, too large. Although carefully measured body weights will usually provide an adequate precaution against overhydration, the data are sometimes more difficult to evaluate if there is a

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weight loss, and at best this information is of value only in terms of the direction of the fluid balance. Comparatively few direct estimations of body water have been made in oliguric patients, and the results obtained with deuterium-[3, 4] and tritium-[5] labeled water or with antipyrine[6, 7] appear to be of a somewhat different order of magnitude from those which have been derived from calculations involving metabolic data.[8] In addition, no direct study has been made of the question whether or not an informative estimate of body water can be obtained from the amount of urea removed and the change in plasma concentration effected by hemodialysis, as was suggested by Black.[9] In the present study, body water has been measured in oliguric subjects with tritiated water, a method that has been well studied in normal subjects and found to be suitable for determination of exchangeable water by dilution disappearance methods.[10, 11] In addition, measurements of body water were made during hemodialysis, utilizing both tritiated water and urea.

Materials and methods

Ten millicuries of tritiated water was administered by vein from 4 to 8 hours before dialysis. The amount used is fivefold larger than that employed by Prentice and associates[10] in their studies on normal subjects, and was required in order to follow H^3 concentrations in the dialysis baths. However, the effect of 3 successive hemodialyses of 100 L. each is to reduce the final plasma H^3 concentration to less than one-tenth the predialysis level. The aliquot of the H^3-water for injection was diluted 1:50,000 for standardization purposes.

Table I. Body water in anuric subjects' predialysis, as determined at several intervals following injection of isotopes

Cases (complete studies)	*1 hour (liters)*	*2 hours (liters)*	*3 hours (liters)*	*4 hours (liters)*	*6 hours (liters)*
H^3 by vein					
1	34.7	36.2	36.4		
2	39.3	39.1	39.6		
3	41.5	45.3	45.6		
4	41.5	44.7	47.4	(46.1)	
5	33.9	35.3	35.8		
6	36.0	39.5	38.4		
7	37.5	39.9	41.5		
8	35.7	36.7	33.9	(36.0)	
9	27.2	28.9	27.5	(28.3)	
10	31.6	30.8	33.4		
11	28.3	30.5	32.5	(30.6)	(31.8)
12	29.6	31.8	33.9	(31.6)	
Other studies					
13 H^3 by gastric tube	41.6	43.7	48.8		
14 H^3 by oral ingestion	37.7				36.4
15 H^3 by vein		40.9	42.8		
16 H^3 by vein		41.0	41.8	41.0	

Three procedures were utilized for the determination of exchangeable body water: (1) Conventional dilution disappearance of tritium-labeled water injected into the blood; (2) plasma disappearance of tritiated body water extracted by hemodialysis; and (3) plasma disappearance of urea extracted by hemodialysis. The conventional dilution disappearance technique[10] usually was carried out 4 hours before dialysis, whereas the 2 extraction disappearance techniques here described were carried out during the course of dialysis. In the case of the dilution disappearance procedure, venous blood samples were taken at hourly intervals, from 1 to 3 hours following the injected dose, with additional samples taken at 4 and 6 hours from a few subjects. In the case of the extraction disappearance procedure, dialysis bath samples were taken at the end of each 2 hour dialysis, and venous blood samples were taken just before and just after each dialysis. One and one-half milliliters of blood or bath samples was isolated by vacuum sublimation and radio-assayed by scintillation spectrometry to a statistical precision of 0.9 per cent according to methods published elsewhere.[11]

Urea nitrogen was measured by the hypobromite method.[12] Plasma water was not measured and the results are expressed as milligrams per 100 ml. of blood or plasma, for the urea data. Each sample was divided into 2 aliquots and deproteinized according to the standard Folin-Wu method. If the results differed by more than 2 per cent, additional determinations were performed on each filtrate; but this was necessary on only one of the specimens. In separate studies in which a modified 1:4 Folin-Wu filtrate is employed, the results obtained with the hypobromite and urease methods[13] have been compared in plasma obtained from 8 different patients and found to agree within 1.5 per cent.

Hemodialysis was performed for periods of 4 to 6 hours on a rotating drum type of Kolff artificial kidney (modified by Brigham).

Calculation.

BODY WATER BY DILUTION DISAPPEARANCE OF H^3-WATER. $W = (I - U)/X$, *(1)* where W = exchangeable body water in liters; I = radioactivity injected, counts per minute (c.p.m.); U = radioactivity excreted, c.p.m. (usually negligible in the oliguric patient); and X = concentration of radioactivity in water sublimated from plasma, c.p.m. per liter.

BODY WATER BY EXTRACTION DISAPPEARANCE OF H^3-WATER. $W = V\ B/(X_1 - X_2)$, *(2)* where V = volume of dialysis bath (liters); B = radioactivity concentration in bath at end of dialysis (c.p.m. per liter); X_1 = radioactivity concentration in plasma water before dialysis (c.p.m. per liter); and X_2 = radioactivity concentration in plasma after dialysis (c.p.m. per liter).

BODY WATER BY EXTRACTION DISAPPEARANCE OF UREA. Equation *(2)* is used, but B represents urea concentration in the bath and X represents urea concentration difference in plasma, before and after dialysis.

Results

Table I shows the results for body water calculated on the basis of blood samples taken at 3 or more consecutive times following one injection of H^3-water by vein. A total of 16 determinations was made by the conventional dilution technique in 12 oliguric patients. When calculated from specific activities of blood samples drawn sequentially in time, other workers have shown that body water determinations follow a saturation type of growth curve where saturation, i.e., "equilibration," is reached in about 2 hours.[10] For this reason it is appropriate to make designed comparisons between hourly time points, as indicated in the analysis of variance shown in Table II, in order to examine how closely equilibration is approached. For the anuric subjects, the analysis shows two useful points: first, aside from the variability in cases, a significant component of the total variability lies in the sampling

Table II. Comparison of variance components for body water determinations in anuric and normal subjects

Data of Table I	*d.f.*	*Sum of squares*	*Mean square*
Anuric			
Sampling times (2 vs. 3 hours)	1	2	2
(1 vs. 2, 3 hours)	1	36	36
Subjects (cases)	11	900	82
Error (discrepance)	22	35	2
*Normal**			
Sampling times (2 vs. 3 hours)	1	9	9
Subjects	10	1,081	108
Error	11	33	3

*This analysis of variance has been carried out on previously published data obtained by us using identical procedures for healthy active adult men; mean body weight 102 Kg; mean exchangeable body water 51 L.[11]

Table III. Vital statistics and blood urea nitrogen on the day body water measurements were performed in patients with acute renal insufficiency

Patient	*Sex*	*Age*	*Oliguria* day*	*BUN mg./100 ml.*	*Body weight (Kg.)*	*Body water (L.)*	*% of Body weight*
L. S.	F	26	8	186	54.9	28.2	51.4
G. S.	F	30	6	239	65.0	35.3	54.3
S. I.	F	26	8	189	69.7	35.6	51.1
R. W.	F	17	13	232	70.1	32.1	45.8
			49	174	61.2	31.5	51.5
W. D.	M	65	7	195	61.7	46.1	74.7
C. W.	M	64	8	230	53.5	32.9	61.5
P. S.	M	76	9	193	65.0	40.7	62.6
L. L.	M	61	10	207	84.0	39.0	46.4
S. K.	M	63	15	146	74.5	41.4	55.6
R. B.	M	84	18	140	69.5	36.4	52.4
W. A.	M	45	9	178	78.6	41.9	53.3
			11	164	77.5	46.3	59.7
W. B.	M	52	9	185	64.7	36.3	56.1
			16	238	64.4	39.4	61.2
			23	287	65.3	45.5	69.7

*The early diuretic phase had just commenced in Patients G. S. and R. B. while in all the other patients the 24 hour urine volumes were less than 400 ml.

times (F ratio = 11.9); second, this difference lies between the first hour and later hours (F = 22.5); and third, discounting variability among cases, the error estimate for procedural factors plus biologic factors inherent in the equilibration process can be seen to be $\sqrt{35/22}$ for anuric and $\sqrt{33/11}$ for normal subjects, or about 1.5 L., since the variances agree reasonably well for the two separate studies.

Table IV. Comparison of exchangeable body water before and during hemodialysis

Case	*Predialysis exchangeable body water by tritiated water (L.)*	*During hemodialysis apparent exchangeable body water*		
		Dialysis interval (hours)	*By urea (L.)*	*By tritiated water (L.)*
1	41.9	2	36.1	29.6
		4	44.1	33.7
		6	48.6	37.5
2	46.3	2	(38.8)	
		4	(44.7)	
		6	(47.2)	
3	36.4	2	36.0	27.2
		4	39.0	27.0
4	36.3	2	31.9	36.7
		4	34.1	38.4
5	39.4	2	30.7	29.0
		4	36.7	31.7
		6	38.7	32.7
6	45.5	2	33.9	31.5
		4	39.0	35.4
		6	42.9	38.6
7	35.3	2	29.4	30.9
		4.5	35.4	35.6
8	28.2	2	(27.5)	
		4	(31.4)	
		6	(34.6)	
9	32.1	2	(29.9)	
		4	(33.1)	
		6	(34.7)	
10	31.5	2	23.1	21.6
		4	28.1	24.6
		6	29.8	27.3
11	32.9	2	25.1	
		4	26.6	24.6
		4.5	32.4	
12	46.1	2	39.2	25.8
		4	46.4	34.5
		6	48.6	37.8
Standard error, predialysis: 1.5				
Mean difference, predialysis less 4 hour dialysis, 9 cases each:			1.6	5.9

The salient data collected in the individual patients are presented in Table III. The body water value is the mean of the second- and third-hour values in all instances except that of Patient R. B., in whom the 6 hour value was used. In the majority of patients, the body water (per cent body weight) was either normal or but slightly increased, definite elevations being observed in only 2 of the cases—W. D., 74.9 per cent, and W. B., 69.7 per cent. It was in the

latter case only that a weight gain, namely 0.9 Kg., was observed in the period preceding any of the body water measurements. It should be noted that despite a fluid balance program, in which replacement was restricted so that the mean weight loss for the group was 0.38 Kg. per day, no low values for body water were encountered. All of these patients were critically ill and all, save possibly the 84-year-old man, exhibited an intense catabolic response. In the 3 patients in whom repeated measurements were made, body water increased absolutely or remained within the experimental error of measurement. Hence it is fair to conclude that absolute changes in body water were reflected accurately in body water referred to body weight (body water per cent body weight).

In Table IV, the predialysis body water values obtained with tritium are compared with values obtained during hemodialysis with urea and tritium. When the hemodialysis data were calculated separately for each bath, large changes in body water values were observed because of the progressively smaller differences in plasma concentration observed with successive bath changes. A more accurate estimation was obtained when the data were calculated on a cumulative basis and are therefore so expressed. It will be noted that with time, progressively larger exchangeable body water values were obtained with both urea and tritium. When the mean values were compared, it was found that the 2 and 4 hour urea figures underestimated the predialysis tritium value by 6 and 1 L., respectively, while the 6 hour urea figure was 1½ L. *larger* than the predialysis value. The hemodialysis tritium values were, with few exceptions, smaller than those obtained with urea and consistently less than the predialysis value; the 2, 4, and 6 hour tritium value understimated this value by 10, 6, and 6 L., respectively.

Discussion

The equilibration of tritiated water in these oliguric patients was essentially complete by 2 hours, which is similar to that seen in normal subjects. The predialysis values listed are the means of the 2 and 3 hour values with no correction being made in the computations for urinary loss of the label, which was trivial in all cases. Since knowledge as to the normal body water is usually lacking in studies done on critically ill patients, the values, for purposes of interpretation, are more readily comprehended when expressed in relation to the body weight. While this expression may be misleading in that percentage values for body water within the normal range will be high for lean and low for obese patients, our interpretation of the results obtained in oliguric patients is that the amount of body water is normal to increased, because the values expressed in terms of body weight are similar to those reported by others, e.g., deuterium,[3, 4] tritium,[5] and antipyrine.[6, 7] Bluemle and co-authors[8] reported somewhat lower values, which were derived from metabolic data, but the differences might be explained in part by the higher fat content of their exclusively female subjects and by the smaller volumes used to replace the insensible water loss. The finding of a normal to elevated body water, the increase in this fraction observed with time during oliguria, as well as the results of Remenchik and colleagues,[6] demonstrating a decrease

in body water during recovery, are all in accord with the evidence indicating that significant additions to the water pool may be made from endogenous sources. The pathophysiologic or clinical significance of an increase in the absolute and/or relative amount of body water cannot be stated based on available evidence. It should be noted further that changes in body weight need not parallel the changes in body water in patients in whom other alterations in body composition are occurring also. In fact, the question of just how closely weight and fluid balance data reflect changes in body water in oliguric patients, warrants further study. Despite an inability to define the optimal level of tissue hydration or how closely the currently accepted replacement volumes approach this ideal, there is ample clinical testimony to the adequacy and prudence of the program suggested by Swann and Merrill,[2] which includes a loss of a small amount, i.e., 0.25 Kg. of weight each day.

In calculating the body water from the data obtained during hemodialysis, the blood samples were taken at the beginning and end of each 2 hour period when the bath was changed and calculated, as mentioned, on a cumulative basis. The blood sample taken at the onset represents a stable concentration in the case of tritium and a relatively stable concentration in the case of urea, while those taken at the end of the first 2 hour period give values which are too low due to incomplete redistribution. This excessively large differential in blood concentration, in turn, gives a calculated volume of exchangeable body water which is too low. As dialysis proceeds and the blood concentration (or specific activity) decreases, this differential becomes smaller and the calculated volumes of body water progressively increase. Although this increase, with the duration of dialysis, in the volume of body water was consistently observed with both urea and tritium, the absolute values were different, with the urea volume almost invariably exceeding those calculated from tritium. This, no doubt, is due to the continued formation of urea during the period of dialysis. Additions to the urea pool apparently become available rapidly since urea distribution throughout body water occurs quite rapidly[14]; thus, and this is particularly striking toward the end of a dialysis when the blood urea concentration has been lowered substantially, significant amounts of urea can be extracted in the presence of comparatively small changes in blood concentration.

In comparing the total body water values calculated from the dialysis data with those determined in the predialysis control period, the urea 4 and 6 hour values were found to be 1 L. less and 1½ L. greater, respectively, than the control value, while all of those determined from tritium extraction were less than the control value by at least 6 L. There can be little doubt that the characteristics of tritium equilibration apply equally to urea equilibration, because (1) urea evidently distributes in the body water[14] and its permeability coefficient in biologic membranes* is practically identical to that of water; and (2) the progressive increase of body water values with duration of dialysis was consistently observed for urea as well as

*Practical permeability coefficients in 10^{-7} mole per square centimeter per second per mole per liter. Water: sheep R.B.C. 0.7; rabbits R.B.C. 1.9.[15] Urea: cattle and man R.B.C. 1.7[16] (recalculated).

tritium, despite differences in absolute magnitude. The somewhat surprisingly close agreement between the predialysis tritium values and the dialysis urea is ascribed to the opposing effects (as outlined above) of incomplete equilibration and an endogenous production.

Edwards,[7] in a similar type of study, has presented data showing that in 10 comparisons in 8 human subjects, the volume of body water calculated from the extraction of creatinine during hemodialysis underestimated by 2 L. the predetermined antipyrine space, findings substantially in agreement with our data. However, exact comparability of the two studies is tenuous since the duration of dialysis, the dialyzer used, the sampling times, and the reference substances were all different.

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The effects of posture on renal function during pregnancy in a patient with diabetes insipidus

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The effect of the supine and quiet standing positions on urine formation has been studied in a near-term pregnant patient with diabetes insipidus. Marked antidiuresis was produced associated with reduced glomerular filtration rate and renal plasma flow, sharp reduction in free water and osmolal clearances, and slight increase in urine osmolality. It is concluded that the antidiuresis resulting from the supine and standing positions during pregnancy can occur independently of antidiuretic hormone.

In pregnancy, venous pooling induced by the supine position or assumption of the upright position is accompanied by antidiuresis and antinatriuresis.[1, 2] One of the mechanisms postulated to explain the antidiuresis is excessive secretion of antidiuretic hormone (ADH). We recently have had the opportunity to test this hypothesis by examining the effect of postural changes on sodium and water excretion in a near-term pregnant patient with diabetes insipidus. Shifting from the position of lateral recumbency to the supine position or to one of standing quietly erect resulted in the usual decrease in urine flow and sodium excretion observed in normal pregnant women at term. Thus, it would appear that ADH is not essential for the development of antidiuresis in response to venous pooling during pregnancy.

Case report

Case No. 140,410. R. J., a 36-year-old housewife, was admitted to the hospital in January, 1958, from the county jail where she was awaiting admission to the State Mental Hospital for hallucinations and bizarre behavior attributed to schizophrenia. Examination

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revealed a semistuporous, disoriented woman with signs of marked dehydration. The serum sodium concentration was 160 mEq. per liter, the blood urea nitrogen was 97 mg. per 100 ml., and the carbon dioxide combining power was 26 mEq. per liter. The diagnosis of diabetes insipidus was suggested by the presence of a urine flow of 2 ml. per minute with a specific gravity of 1.005 at a time when the serum sodium was greatly in excess of 140 mEq. per liter. Following hydration and vasopressin injections serum electrolytes returned to normal and all mental symptoms cleared. Intrinsic renal disease was ruled out subsequently by return of the blood urea nitrogen to 8 mg. per 100 ml., normal phenolsulfonphthalein excretion, and concentration of urine with vasopressin to a specific gravity of 1.018. The brisk response to vasopressin and the absence of any evidence of renal disease, hypokalemia, or hypercalcemia excluded a renal origin for the polyuria. Compulsive water drinking could not account for the polyuria, since copious urine flows with a specific gravity below 1.005 occurred with water restriction and hypernatremia. Therefore, the diagnosis of diabetes insipidus was established. Subsequently the polyuria was well controlled on 2.5 units of vasopressin in oil each day.

The patient reported to the obstetric clinic in July, 1959, during the sixth month of her pregnancy. Her antepartum course had been uneventful; she continued to be well controlled on the same dosage of vasopressin. She was admitted to the hospital at 38 weeks gestation for the current study.

Materials and methods

The study was carried out in the postabsorptive state, after vasopressin had been discontinued for 36 hours. On the morning of the test an oral water load of 1,000 ml. was given. A sustained positive water balance of 1,000 ml. was maintained by the oral administration of water in quantities equivalent to the urinary flow. Suitable priming doses of para-aminohippurate and inulin were given intravenously, followed by a sustaining infusion of each, administered at a constant rate of 5 ml. per minute. After an equilibration period of 60 minutes, three control 10 minute clearance periods were obtained while the patient was in the lateral recumbent position. She was then shifted to the supine position and four additional 10 minute clearance periods were obtained. The lateral recumbent position was then resumed until urine flow returned to the control level. Three additional 10 minute clearance periods were then obtained during quiet standing, following which vasomotor symptoms necessitated discontinuation of the study. Blood samples were collected at the midpoint of each period from an indwelling needle in the antecubital vein. Urine was collected by means of an indwelling catheter in the bladder followed by air washouts. Blood pressure and pulse were monitored throughout the study.

Para-aminohippurate was measured by the method of Bratton and Marshall[3] and inulin by the method of Walser and co-workers.[4] Sodium and potassium concentrations were determined by flame photometry using internal lithium standards. Urine and plasma osmolality were measured with a Fiske osmometer.

Results

The data on the effect of positional change on the excretion of sodium, potassium, water, and solute are presented in Table I. In the supine position urine flow decreased from a control value of 16.6 ml. to 1.4 ml. per minute and was accompanied by a decrease in sodium excretion from 365 μEq to 49 μEq per minute. Potassium excretion decreased from 80 μEq to 18 μEq per minute. The glomerular filtration rate (GFR) and renal plasma flow (RPF) during this same interval fell from control values of 193 ml. and 559 ml. per minute to 70 ml. and 194 ml. per minute, respectively. Quiet standing produced similar changes. It is realized that the recorded values for GFR, RPF, and solute excretion during the supine and standing positions are only approxi-

Table I. Effects of positional change on excretion of water and electrolytes

	Urine					*Clearances*			
Period	*Flow (ml./min.)*	*Na (μEq/min.)*	*K (μEq/min.)*	*Uosm (mOsm./L.)*	*Posm (mOsm./L.)*	*Cosm (ml./min.)*	*CH_2O (ml./min.)*	*GFR (ml./min.)*	*RPF (ml./min.)*
Lateral recumbent position									
1	16.2	340	63	63	276	3.7	12.5	205	564
2	15.6	343	81	73	277	4.1	11.5	204	575
3	16.6	365	80	65	279	3.9	12.7	193	559
Supine position									
4	14.2	235	68	53	279	2.7	11.5	186	586
5	11.2	199	62	65	279	2.6	8.6	144	433
6	5.0	90	30	61	278	1.1	3.9	79	233
7	1.4	49	18	172	275	0.9	0.5	70	194
Lateral recumbent position									
8	19.2	—	—	—	—	—	—	—	—
Quiet standing									
9	19.2	249		40	278	2.7	16.5	207	491
10	8.7	113	46	53	273	1.7	7.0	136	341
11	3.9	86	35	96	273	1.4	2.5	90	305

Uosm = urine osmolality; Cosm = osmolal clearance; CH_2O = free water clearance; Posm = plasma osmolality.

Cosm and CH_2O were calculated as follows: $\text{Cosm} = \frac{\text{milliosmoles per liter of urine}}{\text{milliosmoles per liter of plasma}} \times \text{urine flow.}$

CH_2O = urine flow − Cosm

mations, since dead space errors would be considerable due to short collection periods and rapidly changing urine flows. Even so, there is little doubt that all were reduced considerably.

Associated with the sharp reduction in urine flow was a decrease in both free water and osmolal clearances. Despite these changes urine osmolality increased only slightly, rising to 172 mOsm. per liter during the supine period and 96 mOsm. per liter during the period of quiet standing. This comparatively minor rise in urine osmolality in the face of a drastic fall in urine flow from 16.6 ml. to 1.4 ml. per minute in the supine position and 19.2 ml. to 3.9 ml. per minute during quiet standing is strong evidence that the diabetes insipidus was virtually complete. In addition the instantaneous return of urine flow to 19.2 ml. per minute upon the change from the supine to the lateral recumbent position indicates that there had been no significant release of residual ADH during the preceding periods. Plasma osmolality remained constant throughout the study. The blood pressure and pulse remained unchanged in the supine and lateral recumbent positions, but during quiet standing the blood pressure gradually fell to 80/40 mm. Hg, and was accompanied by an increase in pulse rate. Faintness and nausea appeared at this time, necessitating discontinuation of the study.

Discussion

The role of ADH in the antidiuretic response to venous pooling in pregnancy has not been clearly elucidated. In nonpregnant subjects the antidiuresis

of quiet standing can be inhibited by alcohol,[5] and since alcohol is said to interfere with the release of ADH,[6] the decrease in urine flow has been attributed to augmented ADH secretion. However, Assali and associates[2] have shown that alcohol will not inhibit the antidiuresis of quiet standing in pregnancy. To account for this lack of inhibition they have proposed 1 of 2 explanations. Either the stimulus to ADH secretion elicited by quiet standing during pregnancy is exaggerated such that alcohol cannot overcome the response or the antidiuresis is primarily the result of a fall in GFR.

In this patient with diabetes insipidus, the assumption of the supine position or of one of quiet standing resulted in a fall in GFR and a typical antidiuretic response. Berliner and Davidson,[7] del Greco and de Wardener,[8] and Kleeman and associates[9] have shown that in the absence of ADH a reduction in GFR results in a decrease in solute excretion, a greater decrease in free water clearance, and a consequent increase in urine concentration. In fact, urine concentration may exceed even that of plasma, when GFR is markedly reduced. The finding in this patient of a reduced filtration rate, decreased solute excretion, decreased free water clearance, and a slight increase in urine osmolality confirms their observations and strongly supports the thesis that the antidiuretic response to postural changes in pregnancy is the result of a lower GFR rather than increased ADH secretion.

Addendum. Six months subsequent to the completion of this study the patient died in a mental hospital. Autopsy revealed histologically normal kidneys and marked atrophy of the posterior lobe of the pituitary, with microscopic evidence of gliosis. The cause of death was attributed to pulmonary embolus and diabetes insipidus.

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Effect of liver damage on experimental desoxycorticosterone hypertension in the rat

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Desoxycorticosterone hypertension was produced in 18 rats with pressures greater than 170 mm. Hg without added salt. Four control animals did not receive desoxycorticosterone pellets or CCl_4 injections and had no change in pressure throughout the experiment. An average pressure reduction of 49 mm. was recorded after CCl_4 injections. Two rats, having hypertension of 6 months' duration, responded to CCl_4 in the same manner. This also occurred in metacorticoid hypertension. Serum globulin concentration rose in 15 of 16 animals tested following injection of CCl_4. Morphologically, only fatty metamorphosis seems necessary to cause blood pressure reductions from hypertensive to normotensive levels.

Reversal of experimental renal hypertension has been accomplished by carbon tetrachloride injections in the rat and recovery from the effects of this hepatic toxin has resulted in the return of hypertension.[1] Similar observations have been made on hypertensive patients who later develop liver disease.[2-5] Reversal in their albumin-globulin ratio is associated with persistent reduction of blood pressure.[2]

The present study describes effects of carbon tetrachloride (CCl_4) intoxication on experimental desoxycorticosterone acetate (DCA) hypertension of the rat and compares these with effects of CCl_4 in experimental renal hypertension.[1]

Materials and methods

Sprague-Dawley male rats, weighing 80 to 100 grams, were divided into 18 test and 4 control animals, and fed Purina Lab Chow. Sodium chloride (0.90 per cent) was

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Table I. Blood pressure and serum globulin alterations in DCA hypertensive rats

Animal No.	*Hypertensive period (mm. Hg)*	*Change with CCl_4 (mm. Hg)*	*Serum globulin*	*Recovery period (mm. Hg)*	*Change with CCl_4 (mm. Hg)*	*Serum globulin*	*Remarks*
19	195	–58	↑	185	–58	0	Moderate fatty metamorphosis liver
2	191	–59	↑	182	–50	↑	Moderate fatty metamorphosis liver
5	191	–49	↑	182	–50	↑	Moderate marked fatty metamorphosis liver
1	187	–50	↑	180	–45	↑	Marked fatty metamorphosis liver
21	187	–47	↑	165	–30	↑	Marked fatty metamorphosis liver
17	186	–56	↑	197	–67	↑	Moderate marked fatty metamorphosis liver
11	183	–56	↑	182	–52	↑	Moderate marked fatty metamorphosis liver
23	183	–43	↑	167	–12*		Moderate marked fatty metamorphosis liver
15	182	–35	↑				Moderate fatty metamorphosis liver
18	181	–40	0	170	–40	0	Moderate fatty metamorphosis liver
10	180	–53	↑	165	–45	↑	Fibrosis of liver
16	180	–43	↑	165	–40	↑	Fibrosis of liver
25	176	–46	↑	170	–33	↑	Marked fatty metamorphosis liver
12	172	–52	↑	180	–65	↑	Portal cirrhosis
14	172	–39	↑	172	–57	↑	Marked fatty metamorphosis liver
6	172	–52	↑				Marked fatty metamorphosis liver; additional saline
		(–49 average)			(–46 average)		
A	175	–40					Chronic hypertension, 6 months
B	170	–42					Chronic hypertension, 6 months
28	142	+3	↑	130			Control
30	137	–7	↓	135	0		Control
27	135	0	↑	125	+5		Control
29	132	–2	↑	125	0	↓	Control moderate fatty metamorphosis

*Only three CCl_4 injections given before death in second period. (↑) Denotes rise and (0) denotes no change in serum globulin. Hypertensive period records the average pressure readings 2 weeks before starting CCl_4 injections and the CCl_4 period shows the average pressure during the last 2 weeks of the injections.

substituted for drinking water 2 weeks before the pellet insertion; thereafter, tap water was given ad libitum. DCA hypertension was produced by subcutaneous implants of a wax pellet containing 20 mg. of desoxycorticosterone acetate. Twice weekly the unanesthetized rats were weighed and warmed to approximately 40°C., after which blood pressure was measured by the tail microphone method. When hypertension (blood pressure over 170 mm. Hg) was present for at least 2 months, CCl_4 treatment (0.15 ml. per kilogram undiluted analytic reagent grade subcutaneously twice weekly) was begun. With blood pressure consistently normal (120 to 140 mm. Hg) for 1 month, CCl_4 was discontinued, after which hypertension again appeared. CCl_4 injections were begun again, however, they were now at weekly intervals. Two additional animals were allowed to remain hypertensive for 6 months before CCl_4 injections were begun. The 4 control animals received Purina Lab Chow and tap water and were not made hypertensive, nor did they receive CCl_4 injections. Serum total protein and albumin concentrations were measured by the method of Kingsley[8] during control and test periods. Microscopic sections of the kidneys and liver were made at the end of the experiment.

Results

Of 18 test animals, all developed pressures greater than 170 mm. Hg systolic; to reach this level, one rat (No. 6) was maintained on saline solution rather than tap water following the DCA implant. Pressure reductions (Table I) of more than 35 mm. Hg (average drop: 49) after CCl_4 treatment were recorded. The 4 control animals had no change in their pressures. Animals (Nos. A and B) with DCA hypertension of 6 months' duration demonstrated (Table I) return to normal levels exactly as did those with hypertension of 2 months' duration. Weight loss occurred in 2 test rats (Nos. 2 and 25); the remaining 14 maintained their weight during the initial CCl_4 administration period. Average body weights of test and control groups at the end of the course were 430 and 470 grams, respectively.

Serum globulin concentrations (Table II) rose in 15 of the 16 hypertensive animals tested following CCl_4 administration, and also in 11 of 13 treated the second time with CCl_4.

Fourteen animals survived CCl_4 injections. Their pressures returned to the hypertensive range after cessation of CCl_4. In 2 cases (Nos. 12 and 17) the blood pressure actually exceeded the pretreatment values. Carbon tetrachloride treatment was re-instituted, now at weekly intervals (Table II) because of prior CCl_4 injury. This again resulted in pressure reductions (average drop: 46 mm. Hg). A typical experiment (19D) is represented in Fig. 1.

Table II. Serum protein determinations (grams per 100 ml.)

	Period				
Groups	*Control*	*Hypertensive*	*Liver damage*	*Recovery*	*Liver damage*
Change in serum globulin					
Test group	1.61	+.17	+.45	−.46	+.28
Control group	1.44	+.06	+.24	+.02	−.01
Change in serum albumin					
Test group	4.10	−.17	+.21	+.12	−.22
Control group	4.65	−.09	−.25	+.24	−.84

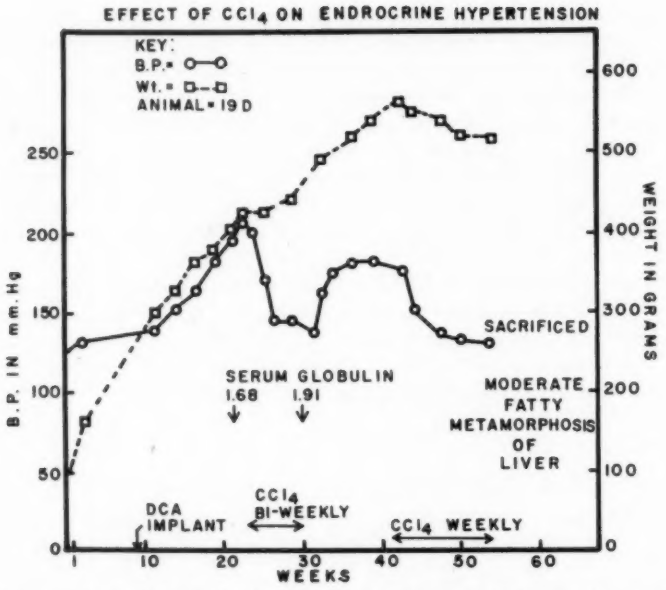

Fig. 1. The changes in weight and blood pressure in a typical experiment following DCA implant and CCl_4 injections.

Only 3 of the test animals developed any degree of cirrhosis on microscopic examination (Table I) and 2 of these showed ascites. The others showed various degrees of moderate to intense fatty metamorphosis. Moderate fatty changes were reported in 1 control rat. Kidneys were reported normal in 8 of the test group; 2 showed only slight hyperemia. Kidneys of control animals were histologically normal. Animals A and B, with chronic hypertension, likewise had only minor changes. Adrenal glands in the examined animals (Nos. 11 and 15) were reported as normal.

Discussion

The results demonstrate that CCl_4 intoxication consistently decreases blood pressure of endocrine (DCA) hypertensive rats to normal levels. The fact that animals with hypertension of 6 months' duration responded similarly shows that this can occur in hypertension of long, as well as short, duration. This was sequentially demonstrated in all 14 animals tested. The degree of liver damage need be only minor (moderate to fatty metamorphosis) to bring about reductions in pressure. Lesions are frequently completely reversible.[12]

Carbon tetrachloride, as administered here, produced no significant effect on the kidneys in 8 of the 14 test animals. The remaining group had only minor microscopic findings of cloudy swelling of the convoluted tubules and hydropic degeneration of the tubular epithelium occurring in the superficial portion of the cortex. These findings are in agreement with those well documented by Smyth and coauthors.[7]

In these experiments, in contrast to the experience of others,[9, 10] persistent hypertension was elicited by DCA without provision of excess sodium or

sensitization by unilateral nephrectomy, although Friedman and associates[14] reported the development of DCA hypertension without added salt. Since the pellets are almost entirely (99.7 per cent) absorbed within 3 months,[10] as was determined in the present study, return of hypertension after cessation of CCl_4 injections, 6 to 7 months after implantation, indicates that a metacorticoid hypertension had been established. This occurred in the absence of the usual renal lesions[11] elicited by giving DCA with a high NaCl intake. This hypertension was also responsive to CCl_4.

CCl_4 treatment resulted in rise in serum globulin concentrations in all but one animal (No. 18); the hyperglobulinemia is attributed to hepatic injury. Our interest in the serum globulin is based on the observation[2] that patients who have prolonged established hypertension, and later develop hyperglobulinemia as a result of liver damage, show persistent decrease in blood pressure. All of the actions of CCl_4 are not completely known and may affect several organ systems beside the liver in lowering blood pressure.

These data show the effect of CCl_4 in producing liver damage and probably a role of the liver in maintaining DCA, as well as renal hypertension.[2] The mechanism of the antihypertensive effect of hepatic injury is unexplained. In dogs, hepatectomy has been shown to abolish formation of renin substrate.[13] It is conceivable that CCl_4 may produce a similar effect in the rat; this is under current investigation.

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The effects of intravenous administration of uric acid on its concentration in plasma and urine of Dalmatian and non-Dalmatian dogs

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The disposition of uric acid has been compared in Dalmatian and non-Dalmatian dogs. Concentrations of uric acid in the plasma during fasting were found to be higher in the Dalmatian dogs. The intravenous injection of uric acid in doses of 40 and 100 mg. per kilogram of body weight led to greater and more sustained hyperuricemia in Dalmatian dogs, even though they excreted a much higher proportion of the administered uric acid. Doses of 40 mg. of uric acid per kilogram of body weight did not lead to apparent renal impairment in either group of dogs, whereas doses of 100 mg. per kilogram produced signs of temporary renal impairment in non-Dalmatian dogs but not in Dalmatians. The smaller dose produced temporary renal impairment in both groups of dogs if some renal insufficiency existed prior to the infusion of uric acid.

Thus, the kidney of the Dalmatian appears particularly well adapted to excrete uric acid, but the Dalmatian hepatic uricase mechanism may not be so efficient as that of other dogs.

Interest in the physiologic mechanisms by which the purebred Dalmatian coach hound disposes of uric acid originated in 1916, when Benedict[1] noted that, in comparison with dogs of other breeds, Dalmatian dogs excrete relatively large quantities of uric acid in the urine. Excessive production of uric acid in Dalmatian dogs was for sometime considered the cause of the observed differences. However, this theory was abandoned in 1948, when Friedman

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*Dr. Duncan was a Wellcome Trust Travelling Research Fellow at the time these studies were carried out.

and Byers[2] demonstrated that the total excretion of purine metabolites (principally uric acid and allantoin) and their total concentration in the plasma were essentially equal in Dalmatian and non-Dalmatian dogs. Uricase was found in the livers of dogs of all breeds, including Dalmatians, but some studies suggested that oxidation of uric acid to allantoin by this enzyme was not as efficient in Dalmatians as in other dogs.[2, 3] Other studies, however, indicated that, in vitro, the rate of oxidation of uric acid by the action of hepatic uricase in Dalmatian dogs did not differ from that observed in non-Dalmatian dogs.[4] Since Friedman and Byers[2] demonstrated that in Dalmatian dogs the renal urate clearance was similar to the glomerular filtration rate, whereas in other dogs 90 per cent or more of uric acid filtered by the glomeruli was reabsorbed by the renal tubules, it has been assumed generally that the only unique aspect to urate metabolism in the Dalmatian dog is absence of tubular reabsorption of uric acid.

However, in Dalmatian dogs, despite the increased rate of renal excretion of urate, the lack of excessive production of urate, and the allegedly normally active uricase, the concentration of urate in the plasma during fasting was reported to be higher, and intravenously injected uric acid was said to be removed more slowly from the plasma in these dogs than in others.[5] We have explored this apparently anomalous situation and have employed for urate determinations the more specific uricase method. Determinations of plasma levels and urinary excretion of uric acid have been performed in purebred Dalmatian coach hounds and in non-Dalmatian mongrel dogs, to each of which uric acid was administered intravenously.

Materials and methods

Uric acid was administered intravenously in amounts of 40 mg. per kilogram of body weight to 4 Dalmatian and 6 non-Dalmatian dogs. One week or more later this experiment was repeated in 3 of the Dalmatian and 3 of the non-Dalmatian dogs. Six months or more after this first study 2 of the Dalmatian and 4 of the non-Dalmatian dogs also received doses of 100 mg. of uric acid per kilogram of body weight. All of these dogs weighed between 7.0 and 12.0 kilograms, except 2 of the Dalmatians, each of which weighed 17.4 kilograms. All dogs fasted for 24 hours before and during the day of the study.

The total dose of uric acid, dissolved in approximately 30 to 60 ml. of warm saturated solution of lithium carbonate, was infused over a period of 30 to 60 seconds. Observations were made on the effect of injection of solutions of lithium carbonate alone on the different breeds. Serial blood samples were taken from the jugular vein before and at specified intervals after the injections of uric acid. Each sample of blood was withdrawn into a heparinized syringe in about 10 seconds; the blood was centrifuged immediately and the plasma was separated.

The concentrations of uric acid in plasma and urine were determined according to the method described by King and Wootton[6] using a purified enzyme.* In several representative experiments the concentrations of uric acid, urea, and creatinine were determined on the same sample of plasma.

The urine from each dog was collected by means of a catheter in the bladder at 15 minute intervals for 1 hour before and 2 hours after infusion of the urate.

*The enzyme used for estimation of uric acid was Worthington Purified Uricase, produced as a highly purified preparation by Worthington Biochemical Corporation, Freehold, N. J.

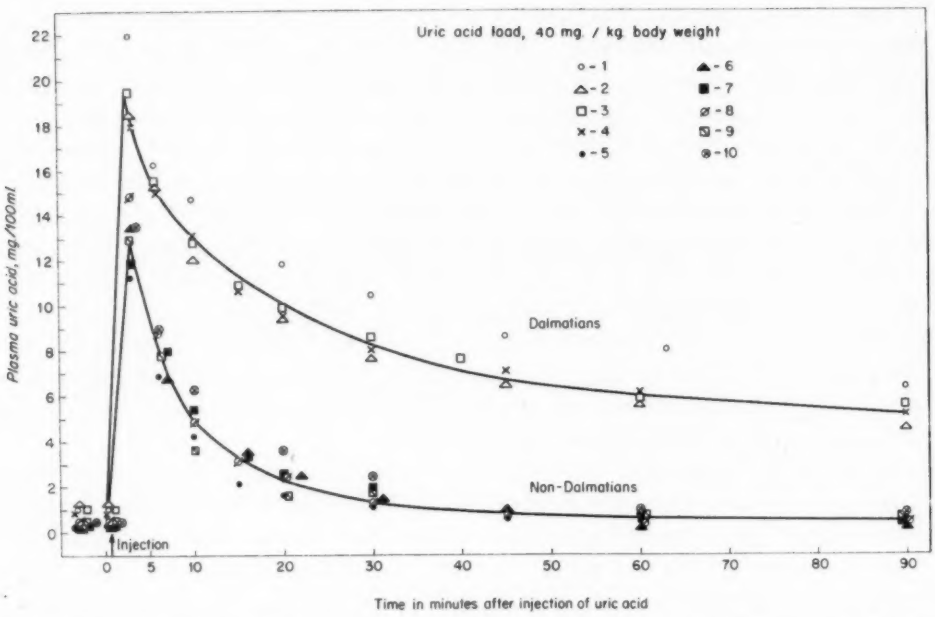

Fig. 1. Plasma uric acid in Dalmatian and non-Dalmatian dogs after intravenous injection of 40 mg. of uric acid per kilogram of body weight.

In all dogs which received the injection of 100 mg. of uric acid per kilogram of body weight a continuous intravenous infusion of 5 per cent glucose was given in order to promote a relatively constant urinary flow.

Femoral arterial blood pressures were recorded on 3 dogs, 2 non-Dalmatian and 1 Dalmatian, all of which received the larger dose of uric acid.

The studies were conducted on conscious dogs during the investigation of the nature and quantitative aspects of renal damage caused by uric acid. The pathologic changes in the kidney will be reported in a separate communication.

Results

Fasting levels of plasma uric acid. Fasting levels of uric acid in the plasma of the 4 Dalmatian dogs ranged from 0.8 to 1.4 mg. per 100 ml., while that of the 6 non-Dalmatian dogs ranged from 0.2 to 0.5 mg. per 100 ml.

Plasma levels of uric acid, urea and creatinine after intravenous administration of 40 mg. of uric acid per kilogram body weight. In Dalmatian dogs the concentration of uric acid in the plasma rose to 18.0 to 21.8 mg. per 100 ml. 3 minutes after the injection and declined to only 5 to 8 mg. per 100 ml. after 60 minutes (Fig. 1). Five hours after injection, plasma urate levels were about 2 mg. per 100 ml. In contrast, in non-Dalmatian dogs the plasma uric acid rose to concentrations of 11.9 to 15.4 mg. per 100 ml. 3 minutes after injection and returned to preinjection concentrations within 60 minutes (Fig. 1). No simple mathematical expression for the rate of fall of the plasma concentrations of uric acid could be derived, but duplicate studies revealed that a fairly reproducible disappearance curve could be plotted.

In 3 of the 4 Dalmatian and in all of the non-Dalmatian dogs no significant changes occurred in plasma concentrations of urea or creatinine after these injections of uric acid. Prior to the injection of uric acid the renal function of all dogs was normal; it remained unchanged after the dose of uric acid.

In the case of the fourth Dalmatian dog, the preinjection renal function of which was 50 per cent normal due to chronic interstitial pyelonephritis which was revealed at necropsy, the response within the first 60 minutes after injection was similar to that of the other Dalmatian dogs; at 160 minutes after injection the plasma urate level was 4.9 mg. per 100 ml. However, at 210 minutes, although concentrations in the other dogs had declined steadily, the plasma urate level in this one Dalmatian dog rose to 5.9 mg., then within 48 hours it declined to 1.1 mg. Plasma urea levels rose from 30 mg. per 100 ml. before injection to 53 mg. 210 minutes after injection and 118 mg. 48 hours after injection. Plasma creatinine rose from the preinjection value of 0.8 mg. per 100 ml. to 1.2 and 2.1 mg. per 100 ml. at 210 minutes and 48 hours, respectively, after injection (Fig. 2).

Plasma levels of uric acid after intravenous administration of 100 mg. of uric acid per kilogram body weight. The rate of disappearance of uric acid from the plasma after injection of 100 mg. per kilogram of body weight (Fig. 3) followed a pattern similar to that observed after the injection of 40 mg. per kilogram of body weight. However, the maximal values were higher and in the non-Dalmatian the return to preinjection values took 90 minutes, at which time the plasma levels of uric acid in the Dalmatians were still 6.9 and 9.3 mg. per 100 ml. Plasma urea and creatinine levels remained unchanged throughout.

Urinary volume and uric acid excretion after urate injections.

INJECTION OF 40 MG. URIC ACID PER KILOGRAM OF BODY WEIGHT. Studies were performed on 2 Dalmatian and 6 non-Dalmatian dogs. In the Dalmatian dogs

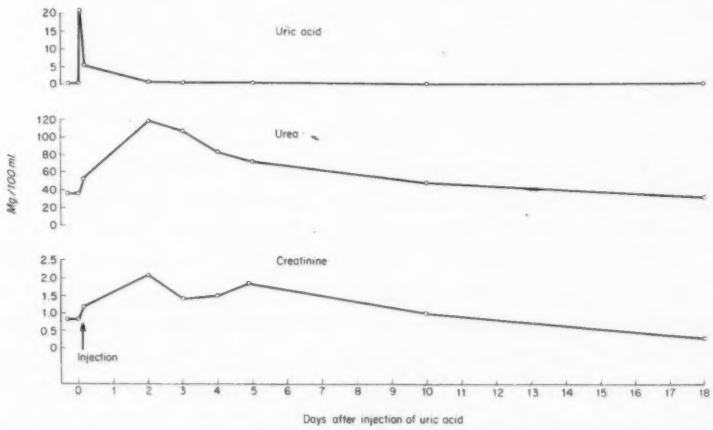

Fig. 2. Serial observations on plasma of Dalmatian dog (No. 1) after intravenous injection of 40 mg. of uric acid per kilogram of body weight.

the volume of urine excreted after the injection of uric acid did not change; approximately 40 per cent by weight of the administered uric acid was recovered in the urine in the first hour after injection, and 12 to 14 per cent more was recovered in the second hour (Table I).

In 6 non-Dalmatian dogs this dose of uric acid (40 mg. per kilogram) did not alter the urinary flow; however, only 11.4 to 18.6 per cent of the uric acid was excreted in the urine in the first hour. Most of the urate which was excreted appeared within the first 30 minutes after injection.

INJECTION OF 100 MG. URIC ACID PER KILOGRAM BODY WEIGHT. Two Dalmatian dogs received this dose of uric acid intravenously. No change in urinary volume occurred after injection. Twenty-two to 28 per cent of the injected uric acid was recovered in the urine in the first hour after injection and 12 to 14 per cent in the second hour.

On the other hand, in the 4 non-Dalmatian dogs which received this larger dose of uric acid, urinary flow was immediately depressed to 30 to 50 per cent of that recorded in the previous hour. Output of uric acid in these non-Dalmatian dogs was 7.7 to 13.6 per cent of the administered dose in the first hour after injection, most of which was excreted in the first 30 minutes. Control injections of lithium carbonate equivalent to those used in conjunction with the uric acid injections of 40 mg. per kilogram did not change the urinary flow; however, those used with injections of 100 mg. per kilogram produced considerable increase in flow. With this larger dose of lithium carbonate the total flow was increased 40 to 60 per cent during the first hour after injection in dogs with constant flow of urine of about 200 ml. per hour. The flow of urine in the second hour after injection was only 10 per cent greater than that measured during the hour previous to injection.

Duplicate studies. Results of the studies in which a second dose of 40 mg. of uric acid per kilogram of body weight was given to 3 Dalmatian and 3 non-Dalmatian dogs were similar to the results of the initial studies.

Studies of femoral blood pressure. Intra-arterial femoral blood pressure was determined before, during, and after injection of 100 mg. of uric acid per kilogram of body weight into 2 non-Dalmatian and 1 Dalmatian dog. The mean blood pressure in all 3 dogs decreased 50 to 70 mm. Hg almost immediately after injection but returned to normal within 3 minutes.

Table I. Uric acid excretion and urinary volume after injection of urate

Breed of dog	*Amount of uric acid administered (mg./Kg. body weight)*	*Additional uric acid excreted (% of administered dose)**		*Urinary volume during first hour after injection*
		First hour	*Second hour*	
Dalmatian	40	40	12–14	Normal
Non-Dalmatian	40	11.4–18.6	0	Normal
Dalmatian	100	22–28	12–14	Normal
Non-Dalmatian	100	6.7–13.6	0	30 to 50 % of normal

*Total uric acid excreted minus amount excreted per hour during control period.

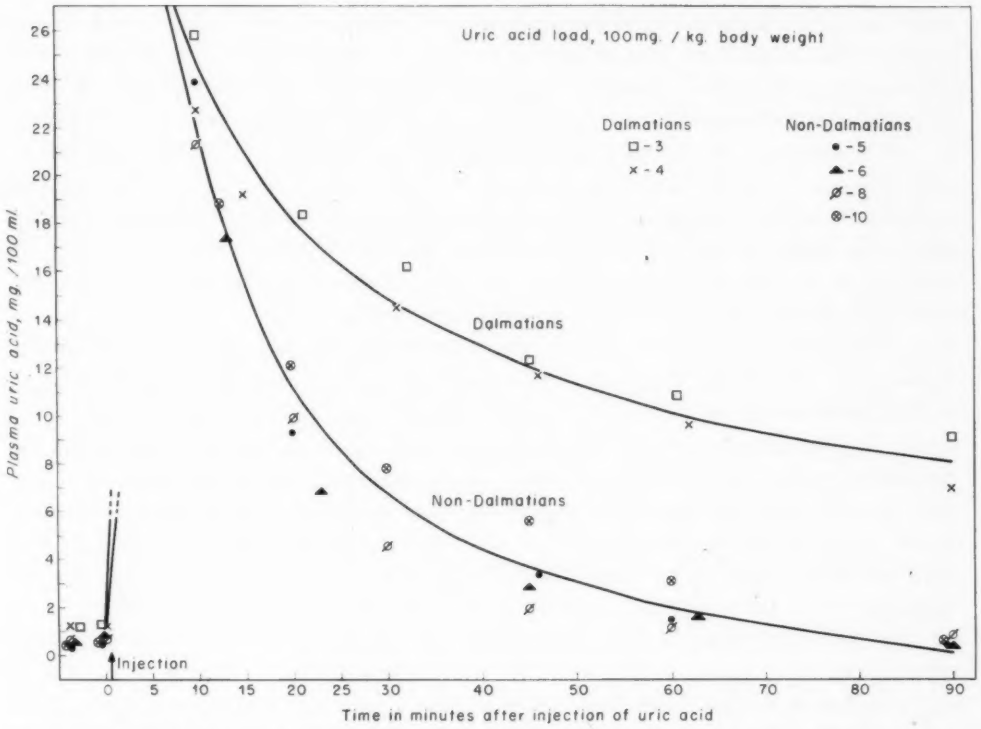

Fig. 3. Plasma uric acid in Dalmatian and non-Dalmatian dogs after intravenous injection of 100 mg. of uric acid per kilogram of body weight.

Comment

The fasting plasma levels of uric acid were consistently higher in the Dalmatians than in other dogs and, after intravenous injection of uric acid, the concentration of uric acid in the plasma increased and remained elevated longer in the Dalmatian breed. At the same time, a greater percentage of the injected uric acid was excreted in the urine of the Dalmatian dogs than in that of the non-Dalmatians. The two major pathways of disposition of uric acid in Dalmatian and other dogs are renal excretion and oxidation by hepatic uricase. The possibility that non-Dalmatian dogs disperse a larger portion of injected uric acid into extravascular compartments is unlikely in view of the studies of Folin and associates,[5] which indicated that "negligible" amounts of uric acid were "stored" in various tissues of dogs after intravenous administration of large doses of uric acid.

Wells,[3] in 1918, demonstrated that Dalmatian liver contained uricase, but his methods did not permit quantitative comparison between the activity of uricase in the Dalmatian and the non-Dalmatian dog. Klemperer and associates[4] later performed studies which seemed to indicate that, at least in vitro, uricase was as active in Dalmatian as in non-Dalmatian dogs. However, Friedman and Byers[2] subsequently suggested the possibility that there was a relative deficit in the conversion of uric acid to allantoin in the Dalmatian dog;

they noted that ligation of the ureters produced a much higher level of uric acid in the plasma of Dalmatian dogs than in other dogs. The results of our studies, as well as the similar results of Folin and associates,[5] provide considerable additional indirect evidence that the hepatic uricase mechanism is not so efficient in Dalmatian dogs. More direct studies of Dalmatian uricase are necessary to confirm this hypothesis. One might also speculate that the excellence of the Dalmatian's renal transport mechanism for disposal of uric acid has lessened its dependence on the use of hepatic uricase, or that the genetic renal tubular abnormality is accompanied by another genetic biochemical defect that influences hepatic uricolysis.

Although our results are in general agreement with those of Folin and associates[5] regarding the variation of concentrations of uric acid in the plasma in Dalmatians as compared to that in other dogs, they are at variance with their results in relation to urinary excretion of urate after intravenous administration to non-Dalmatian dogs. Folin and associates[5] emphasized oxidation of uric acid as the major cause for the rapid decrease in blood uric acid levels after injection because ". . . the elimination of the injected uric acid is so small as to be of no significance." They also stated that because of renal damage ". . . the uric acid excretion in response to the injection of 100 mg. of uric acid per kilogram of body weight is therefore limited to a few milligrams that may come out during the first few minutes." In contrast, during our studies with the same dose of uric acid, from 6.7 to 13.6 per cent of the total administered uric acid was excreted in the urine during the first hour after the injection, by the end of which time concentrations of uric acid in the blood and urine had returned to normal. Thus, it is apparent that the kidney of the non-Dalmatian dog is capable of excreting significant quantities of uric acid when its concentration in the plasma is elevated.

Normally, the non-Dalmatian dog excretes only a trace of uric acid, perhaps 6 to 8 mg. in 24 hours, and this amount probably is derived from the uric acid secreted by the renal tubules, while the small amount of uric acid which appears in the glomerular filtrate probably is reabsorbed and recirculated almost completely.[7]

Intravenous doses of uric acid of 40 mg. per kilogram of body weight did not produce apparent renal impairment in normal Dalmatian or non-Dalmatian dogs, but these doses did lead to evidence of renal impairment in one Dalmatian dog whose renal function was somewhat impaired prior to the administration of uric acid. In other studies we have demonstrated that non-Dalmatian dogs likewise tend to have more extensive renal impairment from intravenous administration of uric acid if their renal function is previously impaired.

It is also interesting to note that hyperuricemia produced by doses of uric acid of 100 mg. per kilogram was not present in the Dalmatian kidney, whereas the non-Dalmatian kidney, under somewhat similar circumstances—indeed with even slightly less hyperuricemia—did exhibit signs of temporary renal impairment manifested by suppression of urinary flow. Histologic examination showed an obstructive type of nephropathy due to aggregation of amorphous urate

and cellular casts in the renal tubules. A more detailed account is to be published later.

These studies on the Dalmatian dog have provided further support to the observation that, in the conditions designated as inborn errors of metabolism, biochemical aberrations are frequently multiple.

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Extraocular muscle, skeletal muscle, and thyroid gland mucopolysaccharide response to thyroid-stimulating hormone

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The effect of thyrotropin (TSH) on mucopolysaccharide concentration of muscle and thyroid gland was studied as part of an investigation of the pathogenesis of the mucinous infiltrate seen in exophthalmos. Extraocular muscle and cervical strap muscle were removed from three groups of dogs. In two groups, one lobe of the thyroid gland was removed at the first operation; one of these groups was given daily saline injections and the other group was given daily injections of 10 units of TSH subcutaneously for 3 to 13 days. The contralateral lobe of the thyroid was removed at the second operation on these animals along with the opposite extraocular muscles and cervical strap muscle. The third group of dogs was similarly handled except that a total thyroidectomy was performed at the time of the first operation and different combinations of thyroxin and TSH were given various subdivisions of this group. Chemical analysis of the tissues of the hemithyroidectomized dogs given TSH demonstrated a significant increase of acid mucopolysaccharide concentration in the remaining lobe of the thyroid gland, averaging 191 per cent by the carbazole method of uronic acid determination and 141 per cent by the orcinol method. Strap muscle showed a significant mean increase of 145 per cent using carbazole and 12 per cent using orcinol. The increase in mean uronic acid values in extraocular muscle was 56 per cent using carbazole and 50 per cent using orcinol; only the increase by the carbazole method was significant. The possibility that

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this effect of TSH on muscle might be, at least in part, extrathyroidal was evaluated in the totally thyroidectomized dogs. A mean increase of mucopolysaccharide concentration in the strap muscle of 90 per cent using carbazole and 69 per cent using orcinol occurred when both thyroxin and TSH were given to these dogs. When either was omitted, or three times the maintenance dose of thyroxin given, no increase occurred.

The pathogenesis of the mucinous infiltrate which occurs in tissues of patients with generalized myxedema[1] has remained in doubt. A similar infiltrate is seen in the skin of patients with hyperthyroidism and exophthalmos who have pretibial myxedema.[2] Chemical[3] and histochemical[4] studies have shown that the infiltrated tissues contain a high concentration of acid mucopolysaccharides, components of the ground substance of connective tissue. In the exophthalmos associated with thyroid disease, there is an increase in the hexosamine concentration of retroorbital tissue, suggesting an increase in mucopolysaccharide concentration.[5] Such a change, with the consequent increase in water binding, could play an important role in the pathogenesis of exophthalmos.

A direct effect of the pituitary thyroid-stimulating hormone, TSH, on tissue mucopolysaccharide may induce these chemical changes responsible for pretibial myxedema and exophthalmos. Asboe-Hansen and Iversen[4] have shown by histochemical techniques that TSH* administration to guinea pigs causes an increase in metachromatic ground substance in retroorbital connective tissue. This report concerns a chemical study of the acid mucopolysaccharide accumulating effect on thyroid tissue, and extraocular and cervical muscles of TSH administered to dogs.

Method

Experimental protocol. Extraocular muscle and cervical strap muscle were removed from three groups of dogs. In two of these groups one lobe of the thyroid gland was removed at the first operation; Group I (6 dogs) was given daily saline injections. Group II was given 10 units of TSH subcutaneously for 3 days (4 dogs), 10 units of TSH daily for 6 and 13 days (2 dogs), 50 units a day for 4 days (1 dog), or 40 units a day for 3 days (1 dog). The contralateral lobe of the thyroid of all animals in Groups I and II was removed at the second operation along with the opposite extraocular and cervical strap muscle.

In a third group of dogs, extraocular and strap muscles were removed in similar fashion, but a total thyroidectomy was performed at the time of the first operation. The dogs in Group III were divided into several subgroups. Group IIIA (5 dogs) received no medication. Group IIIB (5 dogs) received no thyroxin but were given TSH, 10 units daily for 3 days subcutaneously. Ten dogs were given maintenance thyroxin sufficient to maintain a normal serum protein-bound iodine concentration; the dosage was 0.1 mg. per day regardless of the size of the dog. Group IIIC (5 dogs) received the maintenance thyroxin and also received TSH injections. Group IIID (5 dogs) received the maintenance thyroxin alone. Group IIIE (5 dogs) received three times the maintenance dose of thyroxin and also received TSH.

Chemical Analysis. Details of the method of assay of acid mucopolysaccharide used in this study have been published by Bollet.[6] Dried defatted tissues were extracted with 0.5 N

*Supplied for this study as Thytropar by Armour Pharmaceutical Company, Kankakee, Ill.

sodium hydroxide. After neutralization, the protein in this extract was precipitated with perchloric acid. The supernatant solution was dialized and the mucopolysaccharide was precipitated with protamine. The uronic acid content of this precipitate was then determined using carbazole and orcinol methods. Results were expressed in micrograms of uronic acid found in the mucopolysaccharide fraction, per 100 mg. of dried defatted tissue. Repeated analyses of single muscle specimens showed variations never greater than 15 per cent and only once greater than 10 per cent.

Histology. Sections of thyroid gland, strap muscle, and extraocular muscle were taken at each operation. Specimens of thyroid gland were fixed in formalin. Samples of muscle were carefully oriented with the longitudinal axis of the muscle fibers parallel to the fibers of filter paper and fixed in Zenker's solution. Duplicate sections were later stained with alcian blue for acid mucopolysaccharide.

The size of the colloid follicles and the number of mast cells in these specimens were evaluated on coded specimens by two independent observers who had no knowledge of the treatment given each preparation. The results were in close agreement and were averaged.

Results

Chemical observations. The mean values for the change in acid mucopolysaccharide concentration in each tissue of the dogs are presented in Tables I and II. After TSH administration (Group II), the second lobe of the thyroid gland showed an increase in acid mucopolysaccharide concentration of 191 per cent by the carbazole method and 141 per cent by the orcinol method. The thyroid lobes of dogs treated identically but given saline instead of TSH (Group I) showed mean changes of +2 per cent and +7 per cent by these determinations. The differences in mean changes of the values obtained for mucopolysaccharide concentration by the carbazole and orcinol methods in these two groups of dogs are significant. Of the dogs in Group II, which received TSH, the strap muscle showed a mean increase in uronic acid values of 145 per cent by the carbazole method and 129 per cent by the orcinol method of determination; these changes were significantly greater than those found in the saline-treated dogs. The acid mucopolysaccharide concentration in the extraocular muscle increased 56 per cent and 50 per cent but the difference from similar values found in the saline-injected animals was significant only by the orcinol method.

There was no definite correlation between the increase in acid mucopolysaccharide concentration in thyroid gland, strap muscle, or extraocular muscle and the total dose of TSH given, or the period of time over which this dose was administered, when the total dose was over 30 units and the time period was over 3 days.

That the acid mucopolysaccharide accumulating effect of TSH in muscle was not necessarily mediated through the remaining lobe of thyroid gland is suggested by the data obtained in the dogs totally thyroidectomized at the first operation and given maintenance thyroxin and TSH injections (Group IIIC). A mean increase in acid mucopolysaccharide in neck muscle of 90 per cent (carbazole) and 69 per cent (orcinol) occurred compared to minus 10 and plus 6 per cent, respectively, when no TSH was given (Group IIID). The changes in eye muscle mucopolysaccharide concentration, however, were not significantly different in these two groups of dogs.

Table I. Level of acid mucopolysaccharide in dogs in which one lobe of thyroid gland was removed at first operation; data are expressed as the per cent change (i.e., change in mucopolysaccharide concentration divided by level at first operation × 100)

		Thyroid gland		*Strap Muscle*		*Extraocular muscle*	
Group	*Treatment*	*Carbazole method (%)*	*Orcinol method (%)*	*Carbazole method (%)*	*Orcinol method (%)*	*Carbazole method (%)*	*Orcinol method (%)*
I	Saline	− 20	+ 30	− 1	+ 79	− 15	− 2
		− 4	+ 76	+ 5	+ 13	+ 10	+ 22
		+ 18	+ 16	− 8	− 46	− 28	− 20
		+ 7	+ 3	− 38	− 35	+112	+ 1
		+ 47	− 32	− 32	− 36	− 53	− 73
		− 38	− 50	− 11	0	− 45	− 47
Mean		+ 2	+ 7	− 14	− 4	− 3	− 20
SEM*		± 11	± 20	± 7	± 19	± 25	± 14
II	TSH	+102	—	+ 48	+119	+ 19	—
		+ 47	—	—	—	+195	—
		+667	—	+339	+111	+ 35	—
		+359	—	+228	+224	+ 23	—
		+ 74	+218	− 33	+156	+ 3	+ 41
		+111	+128	+340	+170	+ 72	+110
		+ 50	+104	+ 43	+ 72	+ 43	+ 34
		+116	+114	+ 50	+ 52	+ 56	+ 14
Mean		+191	+141	+145	+129	+ 56	+ 50
SEM*		± 74	± 25	± 58	± 22	± 21	± 21
p		<.05.>.02	<.01	=.02	<.01	>.05	<.05.>0.2

*Standard error of the mean.

In view of these data concerning the possibility of an extrathyroidal effect of TSH, it is of interest that no suggestion of acid mucopolysaccharide accumulation in muscle was observed in totally thyroidectomized dogs not given replacement thyroxin, whether or not they received TSH injections (Groups IIIA and IIIB) or in totally thyroidectomized dogs given TSH and three times maintenance thyroxin (Group IIIE).

Morphological observations. No consistent gross differences were observed between the size of thyroid lobes, cervical strap muscles, or extraocular muscles at the two operations on each dog. The total extraocular muscle weight could not be measured because the eye muscles could not be removed quantitatively and because of the difficulty in separating fat and connective tissue from extraocular muscle. This difficulty might also have been responsible for the relatively large variations in acid mucopolysaccharide concentrations found in the eye muscle specimens.

Histologically, as has been previously observed,[7] administration of TSH resulted in approximately a 40 per cent increase in follicular cells and a 40 per cent decrease in colloid in the thyroid gland. The area occupied by stroma remained fairly constant. Thyroid gland mast cell counts increased from 3.5

Table II. Level of acid mucopolysaccharide in dogs in which a total thyroidectomy was done on the first operation; when given, the dose of TSH was 10 units subcutaneously for 3 days, the dose of thyroxin was 0.1 mg. per day, except in Group IIIE which was given 0.3 mg. per day

	Treatment		*Strap Muscle*		*Extraocular muscle*	
Group	*Thyroxin*	*TSH*	*Carbazole (%)*	*Orcinol (%)*	*Carbazole (%)*	*Orcinol (%)*
IIIA	–	–	+ 13	+311	+ 40	+649
			+ 7	+ 13	+ 19	+152
			+ 13	– 36	– 6	– 11
			+ 19	+ 16	0	+ 1
			+ 21	+ 7	0	+ 51
Mean			+ 15	+ 62	+ 11	+168
SEM*			± 2	± 63	± 8	±124
IIIB	–	–	+ 40	+ 67	+ 83	– 5
			0	– 39	– 3	+ 34
			+ 4	– 37	– 12	– 74
			– 18	+ 13	+ 13	– 75
			– 9	+ 2	+ 15	– 12
Mean			+ 3	+ 1	+ 19	– 26
SEM*			± 10	± 6	± 5	± 7
IIIC	+	+	+313	+260	– 23	– 20
			+ 82	+ 45	+176	+ 40
			+ 27	+ 4	+ 39	+ 6
			+ 56	+ 74	– 45	– 2
			– 30	– 38	+ 13	– 24
Mean			+ 90	+ 69	+ 32	0
SEM*			± 13	± 16	± 13	± 3
p†			<.001	n.s.‡	n.s.‡	n.s.‡
IIID	+	–	+ 8	+ 14	+ 39	– 2
			– 28	– 38	– 23	– 29
			– 11	+ 13	+ 22	+ 22
			+ 8	+ 18	– 4	+ 66
			– 28	+ 22	+ 13	– 22
Mean			– 10	+ 6	+ 9	+ 7
SEM			± 3	± 4	± 3	± 5
p§			<05	n.s.‡	n.s.‡	n.s.‡
IIIE	3X	–	+ 48	+ 77	– 9	+ 5
			+ 35	– 28	– 28	– 2
			– 75	– 70	– 36	+ 32
			+ 4	+ 16	+ 18	+ 22
			+ 7	+ 8	– 11	– 5
Mean			+ 4	+ 1	+ 13	+ 10
SEM			± 7	± 8	± 3	± 2

*Standard error of the mean.
†p value for comparison of Group IIIC with Group I.
‡Not significant (i.e., p value > .05).
§p value for comparison of Group IIIE with Group IIID.

mast cells per low-power field in the lobe removed initially to 7.7 per low-power field in the lobe removed after TSH treatment. Mast cells were present in highest concentration around the vessels. No significant amount of interstitial mucopolysaccharide was detected in these sections histochemically.

Strap muscle and extraocular muscle showed no detectable histologic change before or after TSH administration in any group.

Clinical observations. No apparent weakness or exophthalmos developed in any dog. The effect of TSH on exophthalmos could not be determined; a quick-setting dental cement case method for the measurement of exophthalmos was found to be too inaccurate. Totally thyroidectomized dogs without thyroxin medication developed the clinical picture of hypothyroidism with comparable fall in the serum protein-bound iodine values as described before.[80]

Discussion

These data demonstrate that the thyroid-stimulating hormone preparation used is capable of producing an increase in mucopolysaccharide concentration in thyroid tissue and muscle. This increase was greatest in thyroid gland, less in cervical strap muscle, and least in extraocular muscle. We know from previous studies[3] that the degree of increase in the concentration of acid mucopolysaccharide observed chemically in this study does not correlate with histochemical observations. Our data from dogs totally thyroidectomized at the first operation suggest, but do not prove, that TSH can exert an extrathyroidal acid mucopolysaccharide accumulating effect on muscle in the presence of thyroid hormone sufficient to maintain a normal serum protein-bound iodine value.

The TSH preparation used may have been contaminated with other pituitary tropic hormones that conceivably might have exerted these effects. It is of interest that the largest effect of the TSH preparation was exerted on the thyroid gland itself. Although no pituitary tropic hormones other than TSH are known which exert their principal action on the thyroid gland, the lack of purity of the preparation used makes it impossible to rule out an active contaminant. A comparison of the effects of purified TSH and pituitary exophthalmos-producing substances (EPS)[9] on thyroid and extrathyroidal tissues would be of considerable interest and is planned.

The lack of increase in acid mucopolysaccharide in the thyroid gland of dogs injected with saline militates against the possibility that unilateral lobectomy might have resulted in increased endogenous TSH production sufficient to induce the changes observed, unless endogenous and exogenous TSH differ in their effects on mucopolysaccharide metabolism. It is possible that endogenous TSH production might have been increased by unilateral thyroidectomy and was merely quantitatively insufficient to induce the changes observed here, after exogenous TSH. It would be interesting in future experiments to administer propylthiouracil after unilateral thyroidectomy in an attempt to augment endogenous production TSH to a greater extent.

At present it is not possible to draw any conclusions concerning changes

seen in thyroid disease in humans from these data on dogs. The observed effect of TSH on muscle cannot be related to thyrotoxic myopathy because the TSH injected dogs exhibited neither the weaknesses nor the morphologic changes in skeletal muscle which occur in humans. Similarly, it cannot be stated that the accumulation of acid mucopolysaccharide in extraocular muscle under the influence of TSH causes the extraocular muscle swelling observed in malignant exopthalmos, since it was not possible to demonstrate an increase in extraocular muscle volume or that the treated dogs developed exophthalmos.

The lack of effect of TSH on mucopolysaccharide concentration in muscle in totally thyroidectomized dogs given no thyroxin or three times the maintenance dose of thyroxin suggests that the extrathyroidal effect of TSH observed with maintenance thyroxin will not occur in the presence of induced "thyrotoxicosis" or myxedema. It is important to emphasize, however, that dogs totally thyroidectomized and given excess thyroxin differ metabolically from the patient with "malignant" exophthalmos and pretibial myxedema in two important respects. The patient almost always has thyroid tissue present in the neck. He also is thought by many to have a high "set" on the pituitary so that the TSH production is not significantly affected by the administration of thyroid hormone.[10] In addition, clinical data suggest that there is more than one mechanism for the production of myxedema. This tissue change may appear in the presence of hypothyroidism or hyperthyroidism; the infiltrate will disappear when patients with generalized myxedema are given 120 to 180 mg. of desiccated thyroid per day,[1] but administration of desiccated thyroid medication does not improve pretibial myxedema.[2] Therefore, the role of thyroid hormone in regulating tissue mucopolysaccharide concentration needs further study.

Addendum. Since the completion of the studies reported, additional observations have been made using Thytropar which is currently available commercially. This preparation has twice the TSH activity per milligram of protein than does the material which was used in the above studies, because of an undisclosed alteration in the method of processing. This preparation was found to have the same effect on thyroid gland mucopolysaccharide concentration as that reported above, but did not cause an increase in extraocular muscle or cervical strap muscle mucopolysaccharide concentration. These findings support the suggestion that the acid mucopolysaccharide accumulating effect of the TSH preparation used was due to a contaminant rather than the actual thyrotropic hormone.

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Exudative enteropathy. I. A comparative study of $Cr^{51}Cl$ and $I^{131}PVP$

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Quantitative evaluation of the loss of plasma protein into the bowel lumen was compared using 2 radioactive plasma protein tracers, $Cr^{51}Cl$, and $I^{131}PVP$. Each may be recovered quantitatively in the stools after oral ingestion so that significant stool radioactivity after intravenous administration implies intestinal plasma exudation. Acute and slow plasma rates, urinary excretion, and organ localization were compared, and "intestinal clearances" were calculated. In some instances the same patient was studied with both materials. Consistent plasma clearances were not found using either material. However, increased radioactivity was found in the stools of some patients with tropical sprue. Maximum estimated protein losses into the bowel, as calculated from plasma and stool radioactivity, should not have taxed a normal capacity for serum protein synthesis.

Exudation of plasma protein into the intestinal lumen may occur in a variety of conditions grouped loosely together under the term "exudative enteropathy."[1] This diagnosis may be suspected whenever hypoalbuminemia is found without apparent cause. Suspicion is heightened if, in addition, an increased degradation rate of labeled albumin is demonstrable. However, definite proof of this condition is difficult as the wept protein is digested by the intestinal ferments and its constituent amino acids are absorbed to re-enter the amino acid pool.

Recently, the recognition of plasma protein exudation into the bowel has been facilitated by the use of radioiodinated polyvinyl-pyrrolidone (I^{131}-PVP).[1-3] When preparations of suitable molecular size are given intravenously to normal individuals, $I^{131}PVP$ is not excreted into the intestine and, when it is fed, it is neither absorbed from the intestine nor destroyed nor broken down by the intestinal enzymes and bacterial flora. The radioisotope label

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permits its detection in the stool with facility and rapidity. Normally, only very small amounts of iodine are excreted into the stool so that the presence of even modest stool radioactivity after intravenous administration of I^{131} PVP presumably indicates plasma leakage into the intestinal lumen. Hence, a simple diagnostic criterion of exudative enteropathy is available.

Plasma exudation into the intestine may contribute to the hypoalbuminemia of tropical sprue. Severe azotorrhea and an increased apparent degradation rate of iodinated human serum albumin were found in preliminary studies.* As I^{131}PVP was not available to us on initiation of these studies, we sought another protein tag for similar use and undertook certain initial studies with chromic^{51} chloride ($Cr^{51}Cl$). Chromic ion binds firmly to plasma albumin and superficially it appeared to meet the biologic criteria of a plasma protein tracer.[4-6] Moreover, chromic ion is largely nonabsorbable and, although the albumin carrier may be digested, the Cr^{51} would be recoverable in the feces. Subsequently, we have had opportunity† to employ two lots of I^{131} PVP in examining this problem and in some instances could compare the behavior of both $Cr^{51}Cl$ and I^{131}PVP in the same individual. In reviewing our data Dr. Gordon pointed out that the radioiodinated PVP employed in these studies was not identical with the material prepared earlier for studies previously reported.[1-3] Thus our data for either normal or abnormal subjects are not comparable to those of other reports. Most of our data were obtained with our first lot of PVP. The second lot was used mainly for extension and confirmation of our initial observations. A standardized preparation is, of course, critical to comparative clinical observation.

In this paper, the body distribution and excretion of each of these plasma protein tracers are examined in terms of their suitability for the quantitative definition of exudative enteropathy. In a companion publication[7] we report our preliminary clinical experiences with these materials in patients with tropical sprue and other conditions.

Materials and methods

$Cr^{51}Cl$‡ (specific activity: 200 mc. per milligram) was administered shortly after receipt in our laboratory. After brief incubation of this material with plasma, practically 100 per cent of the Cr^{51} activity was precipitable by trichloracetic acid and it appeared that absorption of the chromic ion onto protein was practically instantaneous. Accordingly, no prior incubation was attempted, and 5 to 10 μc of $Cr^{51}Cl$ was injected intravenously. Plasma and whole blood were sampled at intervals over the first 2 hours and daily thereafter. At the end of each study aliquots were counted in a well scintillation detector. Red cell uptake was determined after the first day. Urine was collected daily and the completeness of the collection was monitored by determinations of creatinine excretion.

*By this technique the loss of protein-bound radioactivity from the plasma is assumed to reflect molecular degradation of the labeled albumin within the body. Excretion of whole labeled protein into the bowel or elsewhere outside the exchangeable protein pool would produce the same apparent effect on the plasma curve and, if the iodine is split off by bacterial and enzymatic digestion, reabsorbed, and re-excreted into the urine, it would have the same apparent effect on the retained body radioactivity curve (such artifacts of theory are, in the final analysis, quite real). Such protein losses intensify the net rate of loss of circulating protein and stress the balance between net synthesis and net catabolism of albumin.

†With the cooperation of Dr. Robert S. Gordon, National Institutes of Health.

‡Abbott Laboratories, Inc., North Chicago, Ill.

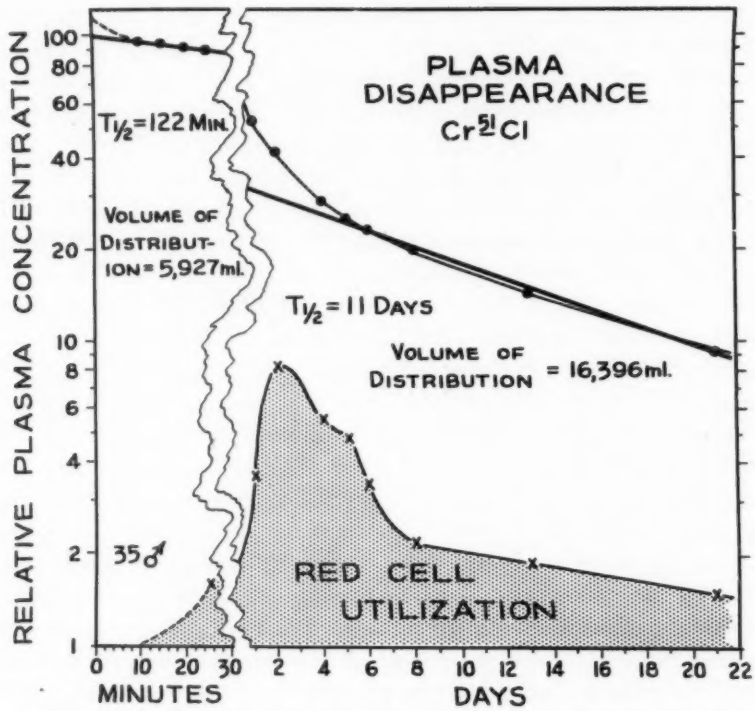

Fig. 1. A typical study utilizing $Cr^{51}Cl$ is shown. Red cell utilization was determined from whole blood and plasma counts and hematocrit levels and it averaged 7.4 per cent dose (S. E. = 1.2 per cent dose) and the slow component volume of distribution averaged 2.4 (S. E. ± 0.4) times the initial volume of distribution.

Stool collections often were facilitated by chronic diarrhea in many cases and markers were not employed. Urine and stools were placed in standard containers and counted, using suitable corrections for geometry. Body localization of retained radioactivity was attempted by external body monitoring using a collimated 2 inch crystal scintillation probe. Sites of greatest radioactivity on the first day's counts were marked in indelible ink and daily counts were obtained over these areas with the detector at the skin surface.

The behavior of $I^{131}PVP$ was examined in similar fashion. Since no radioactivity was found in the red cells, only plasma counts were monitored. Iodide was administered as Lugol's solution to inhibit the thyroid uptake of split I^{131}. Cr^{51} and I^{131} could not be conveniently assayed simultaneously. In those patients who received both isotopes, the second was given only after plasma radioactivity was no longer detectable and excreta and organ radioactivity were minimal and approximately constant.

Results

$Cr^{51}Cl$. $Cr^{51}Cl$ was cleared rapidly from the plasma and several components of decreasing rapidity (Fig. 1, Table I) were evident on inspection of the plasma disappearance curve. The slowest component, usually evident after the fifth day, had a half life of 11 to 16 days. The most rapid component after the initial mixing period had a half time of approximately 70 to 130 minutes. The red cell uptake of Cr^{51} reached a maximum on the second day and thereafter

fell rapidly (Fig. 1). Nevertheless, between the second and fifth days it accounted for a significant portion of whole blood radioactivity. The amount retained in the plasma fell from approximately 50 per cent on the day of injection to approximately 10 to 15 per cent by the tenth day (Fig. 2). Approximately one fourth of the administered radioactivity was excreted in the urine over the first 5 days (Table II). An even greater proportion was retained in some site outside the circulating plasma volume. Little radioactivity was found in the feces of normal subjects (less than 1 per cent of administered dose). Daily fecal excretion in normal persons was relatively constant and usually varied between 0.05 and 0.20 per cent per day. When $Cr^{51}Cl$ was fed by mouth, essentially all the radioactivity was recovered in the feces over a 3 day period. Assuming that an average of 35 per cent of the radioactivity remained in the plasma during the first 3 days, 50 ml. of plasma or 0.5 Gm. of wasted protein would be readily detectable in the stool.

I^{131}. The behavior of $I^{131}PVP$ appeared to differ from that of Cr^{51}. The fast component of the plasma curves after initial mixing was considerably longer than $Cr^{51}Cl$ (Fig. 3, Table I). The slower component was less consistent

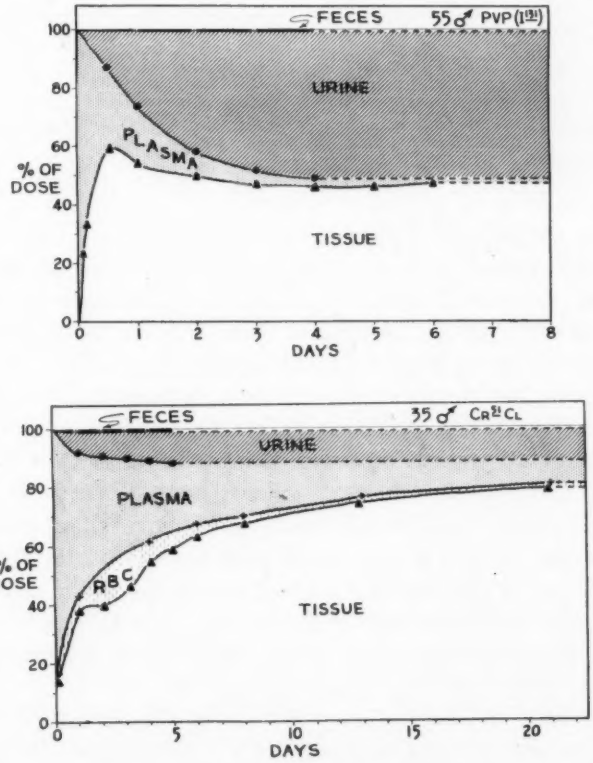

Fig. 2. Body distribution of retained radioactivity is compared in 2 normal individuals. In both studies a large portion of the radioactivity was neither in the plasma volume nor excreted; this unaccounted for portion is arbitrarily designated as "tissue."

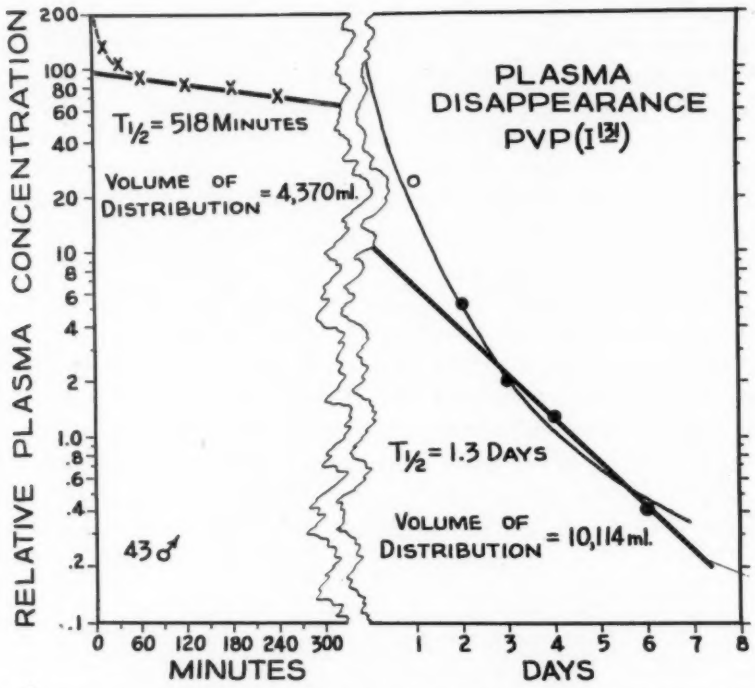

Fig. 3. A typical study utilizing I^{131} PVP is shown. The ratio between immediate and slow component volumes of distribution was 1.9 (S. E. ± 0.3) and not statistically different from the $Cr^{51}Cl$ study.

from patient to patient and had an apparent half life of from 0.5 to 3 days. A third much slower component was apparent in some studies but low levels of plasma activity precluded accurate definition. The amount retained in the plasma fell rapidly; after 24 hours less than 10 per cent of the dose remained and by 48 hours less than 3 per cent. Thereafter plasma radioactivity appeared to fall more slowly, but the total quantity within the plasma volume was usually less than 1 per cent of injected dose (Fig. 2). One half of the injected radioactivity was excreted rapidly into the urine (Table II). Fecal excretion was minimal and generally was less than 0.5 per cent. Fecal radioactivity tended to fall in exponential fashion and generally more than two thirds of the radioactivity found over a 5 day period was present in the first 2 days' stool collection. Because of the rapidly falling nonlinear plasma curve, it was impossible to obtain any consistent estimate of quantitative "intestinal clearance" of the plasma protein even in the cases with increased stool radioactivity.

External monitoring. In the case of both $Cr^{51}Cl$ and $I^{131}PVP$, a large portion of the administered radioactivity was unaccounted for in the plasma, urine, and feces and presumably was retained in the tissues. External monitoring revealed the greatest radioactivity present over the hepatic and splenic areas after either $I^{131}PVP$ or $Cr^{51}Cl$. Despite administration of Lugol's solu-

tion, a relatively large amount of radioactivity was found in the region of the thyroid in some individuals who had received $I^{131}PVP$. The daily relative radioactive uptake of various organs is shown in Fig. 4.

When $Cr^{51}Cl$ was given, little change in the body counts occurred after the first day but, with $I^{131}PVP$, daily counts of the liver revealed consistently changing amounts of radioactivity. A bactrian curve, reaching a peak during the first 24 hours with a secondary rise on the fifth or sixth day, was seen. In some instances, a similar curve was obtained from the splenic counts but this may have been a reflection of hepatic activity.

Discussion

Difficulties were encountered with both plasma protein tags. The initial volume of distribution obtained by extrapolating the slow component curve of either $Cr^{51}Cl$ or $I^{131}PVP$ was consistently greater than a reasonable estimate of plasma volume (range 9 to 32 L.). A major obstacle in the use of Cr^{51} ion was absorption to glassware. This was not minimized by the addition of protein and could not be overcome by the use of a nonradioactive carrier

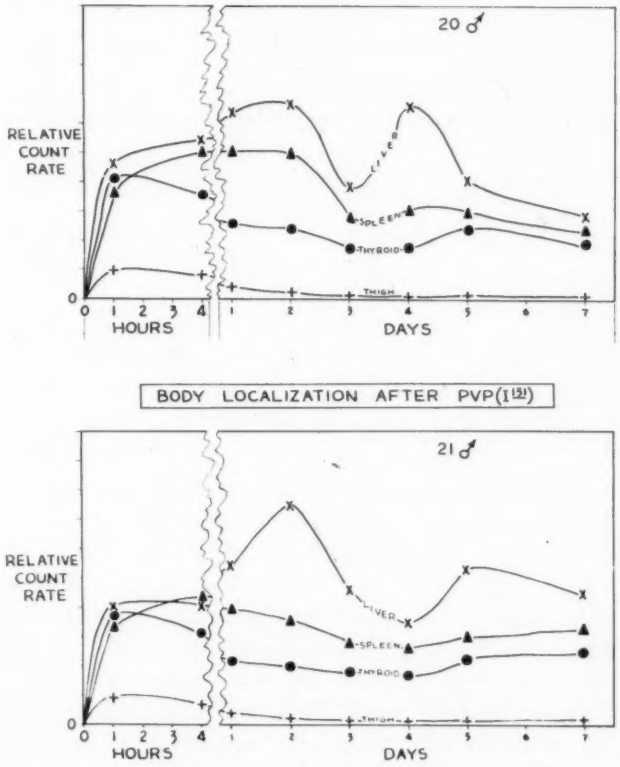

Fig. 4. Two examples of the two-phased liver uptake curves obtained with $I^{131}PVP$ are shown. Note the high thyroid uptake despite the attempted blockade with inorganic iodine.

Table I. Plasma clearance: Apparent half lives of $Cr^{51}Cl$ and $I^{131}PVP$

Case No.	*Fast component (minutes)*	*Slow component (days)*
$Cr^{51}Cl$		
2	132	11.4
6	74	—
9	—	16.1
11	—	10.6
12	106	14.3
$I^{131}PVP$		
2	156	0.9
4	140	2.5
8	195	1.1
10	380	—
12	290	1.7
15	300	—
17	245	—
19	510	1.4

Table II. Urinary radioactivity after intravenous injection of $Cr^{51}Cl$ or $I^{131}PVP$

$Cr^{51}Cl$ (% daily dosage)			*$I^{131}PVP$ (% daily dosage)*	
Case No.	*Day 1*	*Total 5 Days*	*Day 1*	*Total 5 Days*
1	9	19		
2	12	18	41	53
3	21	27		
4	8	9	19	32
5	12	17	38	49
6	22	32		
7	4	16		
8	21	31	45	61
9	16	19	26	50
10	8	16	16	37
11	14	21	31	49
12	10	14		
13			17	28
14			49	62
15			29	43
16			42	61
17			44	53
18			50	76
19			31	51
20			26	44
21			31	49
Mean	13.1	19.9	33.4	49.9
One standard deviation	5.85	6.87	11.09	11.96

The difference between Cr^{51} and I^{131} excretion at either 1 or 5 days is significant at 0.1 per cent level.

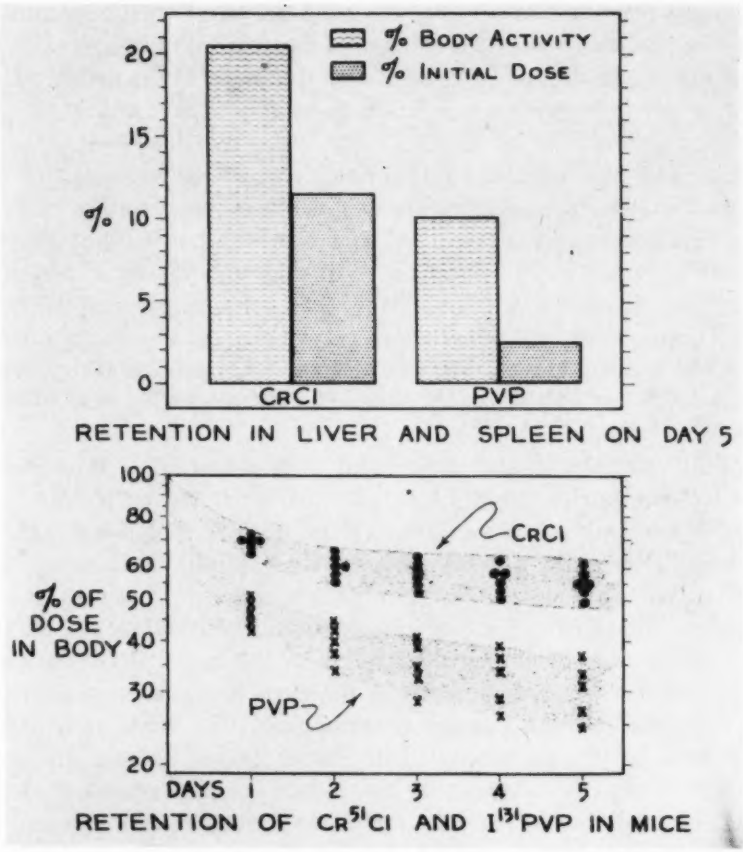

Fig. 5. The greater retention of Cr^{51} ion as compared to $I^{131}PVP$ is reflected in both total body and liver and spleen measurements in mice.

because of the inherent toxicity of chromic ion. Siliconizing glassware or the use of plastic syringes, tubes, and containers tended to reduce absorption but did not obviate it. Each dose had to be standardized separately and assay of dilutions of the standard was often poorly reproducible. Moreover, the long biologic half life of Cr^{51}, coupled with its relatively long physical half life, increases radiation hazards and complicates serial study. However, the larger amounts of radioactivity remaining in the plasma after the intravenous administration of Cr^{51} allowed a more reasonable quantitative measurement of intestinal protein loss.

The main difficulty encountered with the 2 lots of $I^{131}PVP$ was the high content of free iodine (I^{131}) or contamination with iodinated PVP of small molecular size. Following our experience with the first preparation, we learned from Dr. Gordon that more than one quarter of the radioactivity in this lot was freely dialyzable. The second preparation must also have contained appreciable "free" radioactivity for the percentage excreted in the urine was similar for both lots.

Total body retention of $I^{131}PVP$ (second lot) and $Cr^{51}Cl$ in adult mice is shown in Fig. 5. Serial total body counts revealed a difference in handling of the two materials similar to the data for humans. Total body radioactivity fell rapidly after intravenous administration of $I^{131}PVP$, and 60 per cent was excreted during the first 24 hours. Thereafter, the disappearance rate was much slower, but this portion of the disappearance curve was not linear on semilogarithmic plot, possibly because of self-contamination with urine and feces. The disappearance curve of $Cr^{51}Cl$ was similar in contour, although less radioactivity was lost initially and the slow component appeared slower. Five days after injection of the protein tracers both groups of mice were sacrificed. There was 2 to 4 times more total body radioactivity in the group receiving $Cr^{51}Cl$. Also, the proportion of total body radioactivity retained in the liver and spleens of the group given $Cr^{51}Cl$ was twice that of the group given $I^{131}PVP$.

Such data emphasize the two chief difficulties of estimating plasma clearance by comparing fecal loss of radioactivity with any arbitrary estimate of coincident plasma radioactivity. With $Cr^{51}Cl$, fecal radioactivity was changed little while plasma activity was falling, indicating that even frequent diarrhea, as was seen in some patients, does not permit the assumption of tandem samplings of a continuous stream. With $I^{131}PVP$, on the other hand, some patients had more radioactivity in the stools than was present in the entire plasma volume the previous day; an obvious impossibility if the tag were restricted to the plasma compartment and fecal radioactivity reflected a volume of plasma cleared. Integration of the plasma curves and the fecal activity curves (by determining the weights of paper cutouts of the areas subtended) seemed more logical but their quotient often exceeded 1.0. Furthermore, at least in the case of $I^{131}PVP$, a multitude of molecular species was injected, with each molecular size following an individual rate and possibly mode of disappearance from the plasma.

On an empiric basis the amount of stool radioactivity must have some general relationship to the rate at which plasma protein is exuded into the bowel lumen. Such a process may be expected to fluctuate in severity so that both an acute and a longer term variation may be expected even if the problems of passage time through the bowel could be circumvented. The concentration of serum albumin, or better, the quantitative estimates of albumin pool, are vectors of the simultaneous rates of albumin loss and replacement. Yet they cannot be equated to azotorrhea unless it can be assumed that capacity for protein synthesis is normal and no other cause of albumin loss is exaggerated. Finally, in diseases in which the mucosal surfaces are severely compromised, the assumption that no intestinal absorption of whole protein occurs, as an extension of observations in normal individuals, may be in error and introduces further artifact to quantitative estimates. It must be concluded from these data that, with either material, quantitative interpretations of the degree of plasma exudation have very little meaning beyond what is already evident from the plasma albumin content and dietary history.

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Exudative enteropathy. II. Observations in tropical sprue

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The tests for exudative enteropathy discussed in the preceding paper were positive in 6 patients with untreated tropical sprue and a new case of idiopathic exudative enteropathy. They were normal or equivocal in 5 patients with severe hookworm infestation, 4 patients with liver cirrhosis, 5 patients with miscellaneous conditions, and a control subject.

The occurrence of exudative enteropathy in tropical sprue seems to be established, but the significance of the phenomenon cannot be assessed from these observations.

Hypoalbuminemia frequently occurs in tropical sprue and may improve with therapy even though dietary protein is unchanged or reduced.[1] Both dialyzable and trichloracetic-acid-precipitable nitrogen are often increased in the stools of patients with this disease.[2] It may be assumed that protein digestion and absorption are deficient in the untreated patient and improve during therapy. The reduced albumin concentration could be ascribed to a critical diminution of the amino acid pool available for serum protein synthesis. An alternative or supplementary explanation of both hypo albuminemia and azotorrhea would be the exudation of plasma into the gut lumen, termed exudative enteropathy.[3–5] Such protein may be digested to its constituent amino acids and after reabsorption again be available for serum protein synthesis; or if exudation occurs distal to the major site of digestion and absorption, whole protein may contribute to the azotorrhea.

This report describes our preliminary experience with exudative enteropathy in tropical sprue, revealing that this phenomenon does occur in some cases. The data are reported after this limited experience to point out

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the pathologic process; correlation with other features of the disease should be fruitful when additional cases are available for study. We also report a new case of idiopathic exudative enteropathy in a 3-year-old girl. In addition, data are reported from a selected group of patients with severe hookworm infestation, since parasitosis may be a coincidental finding in patients with tropical sprue. For comparison, apparently normal tests are shown in a normal subject, miscellaneous conditions, and liver cirrhosis. Studies on patients with cirrhosis were included as portal hypertension might cause exudation of plasma protein because of back pressure on the intestinal vasculature.

Material and methods

The patients were observed briefly to confirm the referral diagnosis, and then chromic^{51} chloride or ($Cr^{51}Cl$) radioiodinated polyvinyl-pyrrolidone ($I^{131}PVP$) was given intravenously. Urine and feces were collected for 5 days and analyzed by methods described in the previous report.[7]

Idiopathic exudative enteropathy: Report of a new case. A 3-year-old white girl was admitted to Rodriguez United States Army Hospital on Aug. 17, 1959, with "generalized swelling." Four days prior to entry the patient developed periorbital edema followed by the progressive development of generalized swelling. There were no prodromas, and during the 3 month interval preceding the onset of the illness there were no infections, constitutional symptoms, or loss of appetite or weight. The diet had been ample and well balanced. There was no diarrhea or urinary symptoms. The patient had been healthy in the past and had none of the usual childhood diseases. The family history was unrevealing and both parents and 3 siblings were in good health. Physical examination on admission revealed a white girl of normal stature and development. She was alert and moderately active. The predominant and only significant clinical findings were related to the presence of generalized edema. Her face was puffy in appearance and there was moderate periorbital edema. The abdomen was tense and distended and a definite fluid wave was present. The genitals were swollen and all the extremities showed 2 plus pitting edema. No lymphadenopathy or visceromegaly was evident. Cardiovascular, pulmonary, and neurological examinations revealed no abnormalities.

LABORATORY DATA AND CLINICAL COURSE. The hemogram on admission revealed white blood cells, 10,600 per cubic millimeter with a normal differential count; hemoglobin level, 10.2 Gm. per cent; hematocrit level, 35 per cent; and erythrocyte sedimentation rate, 2 mm. per hour. On peripheral smear, the erythrocytes were hypochromic and microcytic in appearance. Urinalysis on admission and daily thereafter revealed no abnormalities. The serum bilirubin level was 1.4 mg. per cent; thymol turbidity, 1 unit; cephalin flocculation, 1 plus after 48 hours; serum cholesterol, 273 mg. per cent; and cholesterol esters, 164 mg. per cent. Total serum proteins initially were 2.95 Gm. per cent; albumin, 1.89; and globulin, 1.06. One week later, total protein was 3.01 Gm. per cent; albumin 1.77; and globulin, 1.24. The serum protein level began to rise slowly and 28 days later was 6.34 Gm. per cent; albumin, 3.86; and globulin, 2.46. Chest x-ray on admission showed the lungs to be clear, but both diaphragms were elevated, presumably as a result of ascites. Radiographic examination of the intestinal tract revealed no evidence of abnormality in the esophagus or stomach. A gross coarsening of the mucosal pattern of the small bowel was noted. This was believed to be the result of either thickened mucosal folds or a large accumulation of mucus within the intestinal lumen. Transit time of the barium was rapid and within 3 hours the small bowel was cleared. The patient was afebrile and relatively asymptomatic throughout the period of hospitalization. The appetite was excellent at all times. The absence of genitourinary, cardiac, and hepatic disease led to a consideration of idiopathic hypoproteinemia.

During the first 2 weeks of hospitalization, the patient received both $I^{131}PVP$ and

Table I. Stool radioactivity following the administration of $Cr^{51}Cl_3$

Case No.	Diagnosis	Dose (%)					
		Day 1	Day 2	Day 3	Day 4	Day 5	5 Day Total
Controls							
1	Psychiatric	0.19	0.12	0.16	0.11	0.12	0.70
2	Cardiac	0.17	0.11	0.18	0.10	*	0.56
3	Fracture	0.12	0.08	0.16	0.12	0.10	0.58
Group I							
4	Hookworm	0.31	*	0.21	0.14	0.12	0.78
5	Hookworm	0.36	0.44	*	0.26	0.14	1.20
6	Hookworm	0.04	0.12	0.14	0.08	0.11	0.49
7	Hookworm	0.12	0.02	0.16	0.11	0.20	0.61
Group II							
8	Cirrhosis	0.12	0.09	0.16	0.12	0.08	0.57
9	Cirrhosis	0.11	0.17	*	0.16	0.12	0.56
Group III							
10	Tropical sprue	0.62	†	1.11	0.42	0.27	2.42
11	Tropical sprue	0.39	0.47	0.31	†	†	1.17+
12	Tropical sprue	0.18	0.20	0.64	0.42	0.32	1.76

*No stool.
†Not assayed.

Cr^{51} Cl. Shortly after completion of these studies, the total protein levels began to rise and the edema subsided with no definitive therapy except a high calorie, high protein diet. Thirty-five days after entry the patient was completely free of edema. Follow-up visits 3 and 6 months later revealed an apparently normal child. Repeat studies employing radioisotopes were not felt justified.

Fecal radioactivity amounted to 2.7 per cent of the administered dose of I^{131} PVP, considerably in excess of normal fecal excretion. Plasma clearance and tissue retention of the isotope in this patient were similar to values observed in normal individuals. When the patient was given $Cr^{51}Cl$, fecal excretion was also excessive. The calculated clearance rate of plasma protein by the intestine was not consistent from day to day; however, the calculated amount of protein lost in the stools after $I^{131}PVP$ was almost twice that calculated after giving $Cr^{51}Cl$. The spontaneous clinical remission that occurred in this patient is particularly interesting but remains unexplained.

Results

Hookworm, liver cirrhosis, and tropical sprue. One normal subject and 20 patients were examined using $Cr^{51}Cl$ or $I^{131}PVP$, or both (Tables I and II). There was no reason to suspect exudative enteropathy in 5 of the patients and they were grouped with the normal subject as controls. Fifteen patients had diseases in which exudation seemed a reasonable possibility: Group I, 5 patients with severe hookworm infestation; Group II, 4 patients with liver cirrhosis; and Group III, 6 patients with untreated tropical sprue.

In the first group, 2 of the 5 patients had excessive radioactivity in the feces. Fecal blood loss in other patients with severe hookworm infestation was only 5

to 50 ml. daily, when measured semiquantitatively by the labeled erythrocyte technique. The smallest amount of plasma which could have contained the radioactivity found in the feces after either I^{131}PVP or $Cr^{51}Cl$ was often more than 150 ml. per day after the first day. This reflects the gross inaccuracy of such calculations based on averaging a changing plasma level and assuming stool collections are complete. Perhaps the excessive fecal radioactivity in the 2 Group I patients represented loss of plasma in excess of whole blood.

The cirrhotic patients, Group II, had no more radioactivity in the feces than the control group. Their hypoalbuminemia was more severe than that of the other patients studied, but it presumably was a result of decreased synthesis since the tests did not support the possibility of exudation. In Group III, fecal radioactivity in each case was greater than in the control group, and in Patients 10, 19, and 20, the increase was particularly striking. There was no evidence of severe parasitosis or intestinal bleeding in any of these patients at the time of study. All had diarrhea and hypoalbuminemia, but the severity of neither correlated well with the amount of radioactivity in the feces.

Discussion

Whether exudative enteropathy contributes significantly to the hypoalbuminemia and azotorrhea in tropical sprue cannot be answered from these

Table II. Stool radioactivity following the administration of I^{131}PVP

Case No.	*Diagnosis*	*Dose (%)*					
		Day 1	*Day 2*	*Day 3*	*Day 4*	*Day 5*	*5 Day Total*
Controls							
2	Cardiac	0.01	0.09	0.08	0.07	0.27	0.52
13	Normal	*	0.00	0.30	*	0.14	0.44
14	Diabetes	0.02	*	0.08	0.00	0.11	0.21
15	Gastrectomy	0.06	0.13	0.02	0.02	0.01	0.24
Group I							
16	Hookworm	0.60	0.31	0.08	0.09	0.02	1.10
4	Hookworm	0.21	*	0.11	0.16	1.10	1.58
5	Hookworm	0.62	0.21	0.14	†	†	0.97+
Group II							
17	Cirrhosis	*	0.12	0.13	0.05	*	0.30
18	Cirrhosis	0.01	0.15	0.05	*	0.04	0.25
8	Cirrhosis	0.02	0.19	0.24	0.10	0.09	0.64
9	Cirrhosis	0.26	0.16	0.15	0.02	*	0.59
Group III							
19	Tropical sprue	3.11	0.66	0.72	0.41	0.63	5.53
20	Tropical sprue	0.19	1.12	0.88	0.70	0.16	3.05
21	Tropical sprue	1.01	0.21	0.11	0.32	†	1.65+
10	Tropical sprue	0.66	1.12	0.41	0.37	0.29	2.85
12	Tropical sprue	0.77	0.70	0.20	*	0.10	1.77+

*No stool passed.
†Stool passed but not collected.

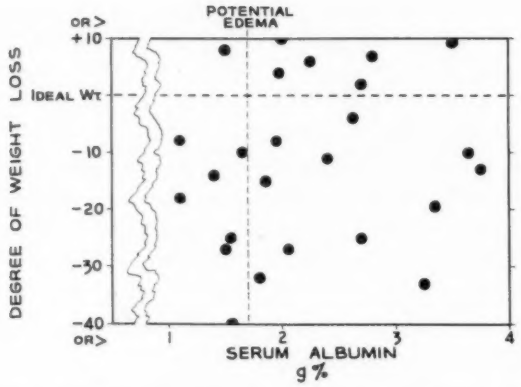

Fig. 1. Relationship of hypoalbuminemia to weight loss in tropical sprue.

studies. Quantitatively the amino acids derived from plasma weepage would be small as compared to the amount ingested and some of the products of protein digestion are undoubtedly reabsorbed to be available again for albumin synthesis. More important is the loss of circulating plasma protein outside the normal process of degradation and, hence, increasing albumin turnover.

Exudation of plasma protein into the gut would tax the capacity for albumin synthesis already limited by inadequate intake and probably deficient digestion and absorption of protein. One may anticipate that hypoalbuminemia in tropical sprue may be more severe than that predictable from simple caloric and protein malnutrition and that the apparent degradation rate of labeled serum albumin may be increased. Correlation of hypoalbuminemia and weight loss in 25 untreated patients was low (Fig. 1). The half life of I^{131} human albumin in 3 patients with tropical sprue was 8.0, 10.4, and 9.8 days, values somewhat less than that obtained in normal individuals. However, albumin turnover, i.e., the product of disappearance rate and exchangeable albumin pool, was not increased.

The few cases of hookworm infestation studied do not permit any broad generalizations. That some patients should show slightly increased stool radioactivity is not surprising and points to another way whereby a heavy worm burden which is tolerated with relative impunity by the well-nourished patient could produce debilitating illness in persons in the lower economic strata. Azotorrhea and hypoalbuminemia have been noted in some patients with hookworm infestations, and, as might be anticipated, usually those with the heaviest infestation.[6] But, as in sprue, deficiencies in the diet and in the digestion and absorption of protein are likely.

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Abnormalities of tryptophan metabolism in a patient with malabsorption syndrome

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Three distinct abnormalities of tryptophan metabolism were observed in a patient with a complex malabsorption syndrome. An unusual urinary excretion of indolic compounds was present which was curtailed by neomycin sterilization of the intestine. Excessive urinary 5-HIAA excretion was found only when intestinal hypermotility and diarrhea were present; the origin of this appeared to be an increased amount of tryptophan metabolized along the 5-HIAA pathway and the stimulus for the latter may have arisen in the hypermotility of the intestine. Finally, a block in tryptophan metabolism, related to apparent deficiency of vitamin B6 coenzyme, was detected and corrected following administration of the vitamin. Therefore it appears that intestinal bacterial metabolism, intestinal hypermotiliy, and vitamin B6 deficiency were each responsible for a particular abnormality of tryptophan metabolism. In a manner of speaking, these metabolic abnormalities appeared as artifacts of the primary disease, namely, the malabsorption syndrome. In their own right, however, the metabolic abnormalities may have significance for the well-being of the patient.

Abnormalities of tryptophan metabolism can occur in certain malabsorption syndromes. Elevated urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA) has been reported in nontropical sprue,[1] tropical sprue,[2] and Whipple's disease[3] but not in several other diarrheal disorders.[1, 4] An elevated urinary indolyl-3-acetic acid (IAA) excretion may also be detected in idiopathic sprue.[2, 5] In addition to these particular biochemical observations, fat- and water-soluble vitamin deficiencies and protein malnutrition can occur in malabsorptive states which in turn may affect enzyme activity at the kynureninase stage in tryptophan metabolism[6]; this may occur because of the vitamin B6 coenzyme requirements of kynureninase or because of interference with syn-

This study was carried out during tenure of a McLaughlin Travelling Fellowship, 1958-1960, at the Medical Unit, University College Hospital Medical School, London, England.
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thesis of this protein apoenzyme.[7] This paper describes all three biochemical abnormalities of tryptophan metabolism occurring in a patient with a severe malabsorption syndrome.

Case history

The patient, a white woman, was 46 years old at the time of the present investigation. She has been the subject of a previous report[8] describing hyperparathyroid adenomas complicating a chronic malabsorption syndrome which at that time (1956) was considered to be adult celiac disease. A gluten-free diet had been prescribed but the patient did not adhere to the diet. In 1958, prolonged exacerbation of steatorrhea occurred producing severe malnutrition. Recovery of relatively normal nutrition and bowel function followed therapy with prednisolone (dosage 40 mg. daily, eventually reduced to 12.5 mg. daily). A gluten-free diet was again prescribed under close supervision and the patient complied with instructions. Despite this regimen, remission lasted only 6 months; steatorrhea, malnutrition, and edema eventually ensued. On readmission to University College Hospital in October, 1959, hypoalbuminemia and macrocytic anemia were present. Extensive skin lesions with some of the characteristics of riboflavin deficiency were present; riboflavin administered orally had no effect on the dermatitis and resolution of the lesions suggestive of riboflavin deficiency occurred only after parenteral multivitamin administration. Excessive borborygmi and frequent watery, bulky stools suggested intestinal hypermotility. The biochemical studies reported in detail were carried out following correction of the riboflavin deficiency.

The condition of the patient steadily deteriorated and she died on Jan. 8, 1960. At necropsy, reticulum cell sarcoma was found. Gross examination revealed apparent extensive involvement of all major lymph nodes, including those in the abdominal cavity and mesenteries; unfortunately the mesenteric nodes were not examined microscopically to ensure that they were also affected by the malignancy noted in other nodes.

Special studies

The following investigation was performed with the intent of defining certain characteristics of the malabsorption state not usually evaluated.

Materials and methods. Tryptophan metabolism was studied indirectly by chromatographic examination of indolic constituents and amino acids in urine. Precautions were taken to overcome the hazards in interpretation of endogenous tryptophan metabolism by this method.

1. The chromatograms were standardized by application of a urine volume equivalent to 1 mg. total nitrogen (micro-Kjeldahl) for "indoles" and equivalent to 250 μg total nitrogen for amino acids.

2. Serotonin-containing foods[9] were eliminated from the diet.

3. Intestinal bacterial production of indolic compounds was suppressed by administration of neomycin orally (0.5 Gm. every 6 hours).

4. Critical studies of indolic acid excretion require control of factors affecting nonionic diffusion[10]; this was not considered essential for this particular study.

Loading with DL-tryptophan orally (35 mg. per kilogram) was employed to increase the chances for detection of an abnormality of tryptophan metabolism. Analysis of a 24 hour control urine specimen preceded amino acid loading. DL-tryptophan was then administered to the fasting patient at 8 A.M. and 3 consecutive 8 hour collections were made thereafter. Urine collections were protected from ultraviolet light under cold acidic conditions (3 c.c. concentrated HCl in a bottle which stood in an ice bucket) and frozen immediately upon completion of collection.

Urinary indolic excretion was studied on the third or fourth day of each of the fol-

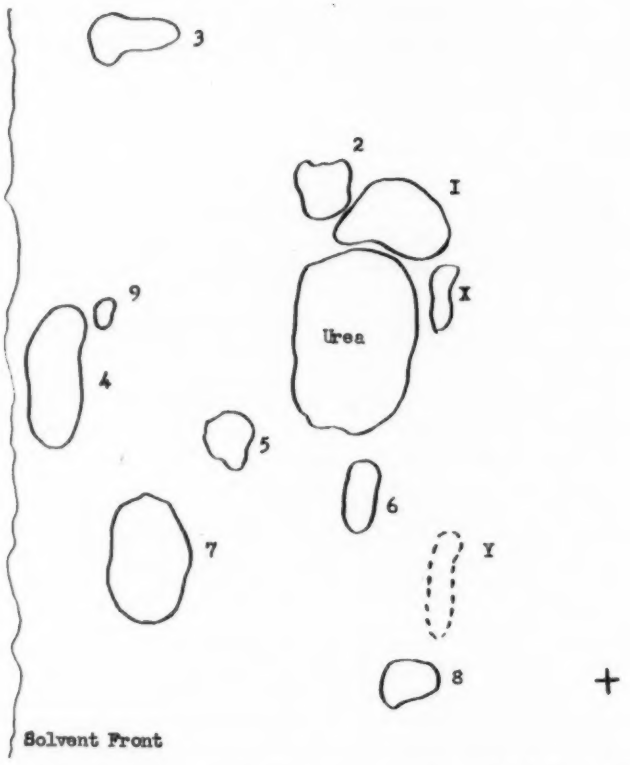

Fig. 1. Untreated state (24 hour urine). Excessive excretion of indolic acids, indole derivatives, and 5-HIAA. Riboflavin is from therapeutic administration. *1,* indoxyl sulfate; *2,* 6-hydroxyskatole sulfate (probably); *3,* Indole acetamide; *4,* indolyl-3-acetic acid; *5,* Indole acetylglutamine; *6,* tryptophan; *7,* 5-hydroxyindoleacetic acid; *8,* Riboflavin; *x* and *y,* unknown compounds; 9, indolyl-3-lactic acid.

lowing regimens: A, normal diet; B, normal diet and oral neomycin; C, oral neomycin and administration of pyridoxine HCl as 1 per cent ointment in water-soluble base, 20 Gm., 4 times daily to the left half of the body (this regime was chosen to determine whether vitamin B6 would also have any direct effect on the dermatitis); D, fruit and vegetable restricted diet without added neomycin.

Three normal adults served as controls for regimens A and D with DL-tryptophan loading. A patient with Hartnup disease[11] who was being studied at the same time provided a comparable study for the effect of neomycin administration on abnormal urinary indole excretion.

Analytical methods. Indolic compounds were identified by two dimensional filter paper partition chromatography according to Jepson.[12] Qualitative comparison of urinary indolic excretion patterns under different conditions was the primary objective. A semiquantitative assessment was obtained by visual grading of spot intensity. Amino acids were identified on filter paper by a combined high-voltage ionophoretic and partition chromatographic method.[13] 5-HIAA was measured semiquantitatively, with greater sensitivity by running replicate chromatograms against measured 5-HIAA standards and comparing resultant spot intensities (sensitivity ± 20 per cent). Total IAA was measured by the chemical method of Weissbach and co-workers.[5]

Results

The data are summarized in Table I and in Figs 1 through 4 which are composite tracings of the various chromatograms produced with several location agents in each study.

Untreated state. A heavy urinary indole excretion was detected in the 24 hour urine collection (Fig. 1). The abnormal excretion included large amounts of indoxyl-3-sulfuric acid, IAA, alpha-N-indolyl-3-acetylglutamine, indolyl-3-acetamide 6-hydroxyskatole sulfuric acid (not positively identified), and 5-HIAA. Unidentified compounds were also present.

The excretion of 5-HIAA was estimated to be 20 mg. per 24 hours (normal: less than 9 mg. daily[9]). IAA excretion was estimated to be 30 mg. per 24 hours (normal: less than 18 mg. daily[5]).

The amino acid chromatogram revealed normal urinary amino acid excretion.

Effects of oral neomycin administration. The excessive excretion of indole and indolic acid derivatives was greatly reduced (Fig. 2, Table I). The urinary 5-HIAA excretion remained unaltered (approximately 18 mg. per 24 hours).

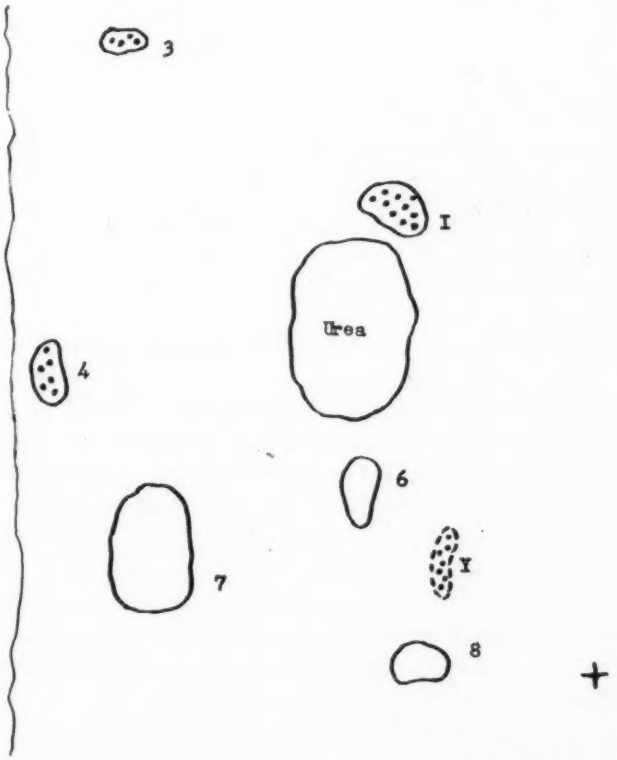

Fig. 2. Effect of neomycin administration (24 hour urine). Excretion of indolic acids and indole derivatives greatly diminished (stippled spots); 5-HIAA unaffected.

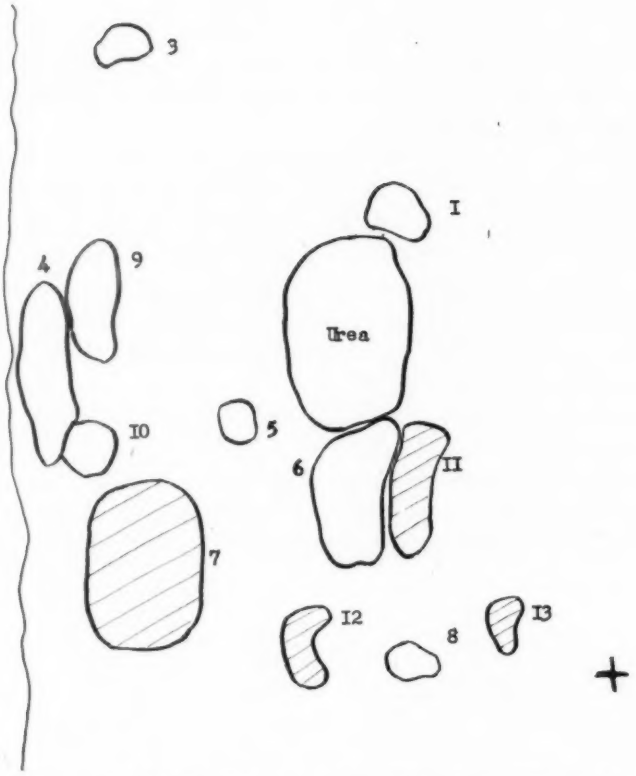

Fig. 3. First 8 hour urine following DL-tryptophan (35 mg. per kilogram) orally (neomycin continued). Evidence for: block in tryptophan metabolism with excessive excretion of xanthurenic acid, kynurenine, and probably of 3-hydroxykynurenine; and 5-HIAA "shunt" (excretion of 5-HIAA is at least 100 per cent above control base line). Hatched spots represent abnormally large excretion; *9,* indolyl-3-lactic acid; *10,* anthranilic acid (probably); *11,* kynurenine; *12,* xanthurenic acid; and *13,* 3-hydroxykynurenine (probably).

DL-tryptophan loading during neomycin administration. The following results were observed.

1. The 8 hour urine collection, immediately following DL-tryptophan loading, contained a distinct increase in 5-HIAA compared to the control specimen (compare Fig. 3 and Fig. 1, respectively); this increase in 5-HIAA excretion was estimated to be at least 100 per cent. The normal subjects showed no detectable increase in 5-HIAA excretion following loading with 35 or 70 mg. per kilogram doses of DL-tryptophan. A loading dose of 100 mg. per kilogram L-tryptophan (equivalent to 200 mg. per kilogram DL-tryptophan) is required to produce a significant increase in 5-HIAA excretion.[5]

2. There was an excessive urinary excretion of xanthurenic acid (XA) immediately following DL-tryptophan loading (Fig. 3); at the same time, kynurenine and 3-hydroxy-kynurenine* excretion seemed excessive.

*It was not possible to perform replicate chromatograms with a marker of pure 3-hydroxykynurenine. Identity rests only on position of the spot, staining characteristics, and its disappearance following pyridoxine administration.

3. Tryptophan and its endogenously produced metabolites (e.g., kynurenine and XA) were excreted maximally in the period immediately following oral loading. The remaining collection periods in the 24 hours following loading showed declining urinary excretion of tryptophan and its metabolites. These results were verified in a second loading test.

Toxic symptoms comparable to those observed in patients given tryptophan in conjunction with a monoamine oxidase inhibitor[14] were absent in this patient.

Effect of vitamin B6 administration (neomycin continued). Pyridoxine-HCl applied to the left half of the patient's body did not improve the dermatitis on either half of the body. However, DL-tryptophan loading on the fourth day of the pyridoxine and neomycin regimen was not followed by excessive excretion of xanthurenic acid or 3-hydroxykynurenine; kynurenine excretion appeared to be less than in the loading study before pyridoxine administration (Table I).

Effects of decrease in bowel hypermotility. Introduction of the fruit and vegetable restricted diet was immediately followed by a dramatic improvement

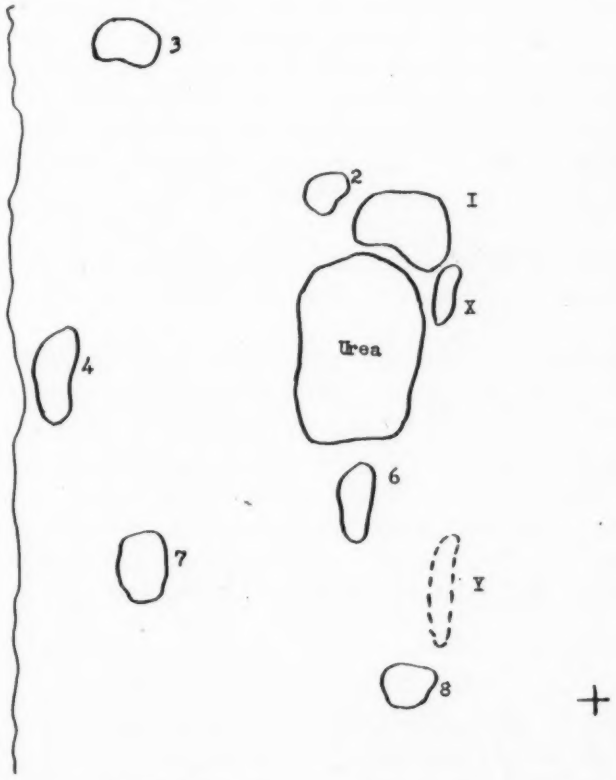

Fig. 4. Effect of decreased intestinal hypermotility; neomycin omitted (24 hour urine). 5-HIAA excretion practically normal. Exogenous (intestinal) indolic compounds again accumulating in excess following withdrawal of neomycin.

Table I. Paper chromatography of urinary indole derivatives: patient and control subjects

Regimen	Collection period (hours)	Principal urinary indole derivatives*													
		Try	*IXS*	*SKS*†	*IANH₂*	*IAA*	*IAGNH₂*	*5-HIAA*	*ILA*	*AA*	*Kyn*	*3-OHKyn*	*XA*	*X*	*Y*
Patient															
Ordinary diet	24	2g‡	4s	3g	2g	3S	2g	3g	1tr	0	0	0	0	*2g*	*2g*
Ordinary diet plus neomycin	24	2g	2g	0	1f	<2f	0	3g	0	0	0	0	0	*0*	*1f*
Ordinary diet, neomycin, DL-tryptophan	0-8	>4vs	2g	0	1f	4vs	2f	>4s	3S	2f	3vs	1g	2s	0	0
	8-16	4g	2g	0	1f	3g	0	4g	2g	0	3g	–	2f	0	0
Ordinary diet, neomycin, DL-tryptophan, plus pyridoxine	0-8	>4vs	2g	0	1f	4vs	2f	>4s	3S	2g	3g	0	0	0	0
Restricted diet	24	2	3S	1g	2g	2g	0	<2f	0	0	0	0	0	1f	2f
Controls															
Normal, ordinary diet	24	2f	3g	0	1f	1f	0	<2tr	0	0	0	0	0	0	0
Normal ordinary diet, DL-tryptophan (70 mg. per kilogram)	0-8	>4vs	3g	0	1g	4vs	1f	<2tr	3s	2s	3g	0	0	0	0
	8-16	3g	3g	0	1g	3s	1f	<2tr	2f	2f	2g	0	0	0	0
Hartnup,[18, 19] ordinary diet	8, fasting	3S	4s	2g	1tr	2f	1tr	<2tr	0	0	1f	0	0	0	0
Hartnup, restricted diet plus neomycin	8, fasting	3S	3g	0	0	0	0	2tr	0	0	1f	0	0	0	0

*Nomenclature and abbreviations after K. N. F. Shaw[18]: Try, tryptophan; IXS, indoxyl-3-sulfuric acid (indican); SKS, 6-hydroxyskatole sulfuric acid; $IANH_2$, indolyl-3-acetamide; IAA, indolyl-3-acetic acid; $IAGNH_2$, α-N-indolyl-3-acetylglutamine; 5-HIAA, 5-hydroxyindoleacetic acid; ILA, indolyl-3-lactic acid; AA, anthranilic acid; Kyn, kynurenine; 3-OHkyn 3-hydroxy kynurenine; XA, xanthurenic acid; X and Y (unidentified compounds): X, fast reacting green-blue with Ehrlich's aldehyde reagent, Y, red-brown with diazotized sulfanilic acid reagent.

†Identification of this compound may be in doubt.

‡Numbers represent average spot size in ¼ inch units. Letters represent visibility: tr, barely visible; f, faint; g, good; s, strong; vs, very strong.

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in frequency and bulk of bowel movements and diminished intensity of borborygmi. This was interpreted to represent a decrease in bowel hypermotility although the reason for this remains unclear. Neomycin was discontinued after introduction of the diet.

The urinary excretion of indolic compounds on the third day of this regimen revealed two alterations: there was an increment in the excretion of IAA, indoxyl-3-sulfate, and their related compounds (Fig. 4); the excretion of 5-HIAA had diminished to 10 mg. per 24 hours.

AMINO ACIDS. There were no significant abnormalities in the urine amino acid excretion detected during the course of these studies. A decline in the patient's condition prevented any further investigation shortly after the completion of regimen D on Dec. 15, 1959.

INTERPRETATION OF RESULTS. The response to neomycin administration, and later to its withdrawal, suggests that, with the exception of 5-HIAA, the excessive urinary 'indole'' excretion had its origin in excessive amounts of intestinal intraluminal tryptophan, with subsequent production by intestinal bacteria of indolic compounds which were then absorbed from the intestinal lumen and eventually excreted in the urine.

On the other hand, failure of the abnormal urinary 5-HIAA excretion to respond to neomycin administration suggests that this abnormality was not dependent on intestinal bacterial action. The abrupt decline of 5-HIAA excretion following control of intestinal hypermotility suggests that hypermotility itself could have been responsible for the excessive excretion. Furthermore, the DL-tryptophan loading data indicate the presence of a shunt augmenting endogenous serotonin-5-HIAA production which was probably operative only during intestinal hypermotility. The rate of the excretion of free tryptophan and its endogenous metabolites following oral administration indicate that there is normal intestinal transcellular transport of DL-tryptophan.

Urinary excretion of large amounts of XA (in association with increased amounts of kynurenine and 3-hydroxykynurenine) following loading with tryptophan is a sensitive index of vitamin B6 deficiency[15]; this particular observation reflects inhibition by coenzyme deficiency of the apoenzyme, kynureninase.[6] The appearance of XA excretion and its disappearance following pyridoxine administration suggest that vitamin B6 deficiency complicated the patient's malnutrition. The response to pyridoxine administered on the skin corroborates previous studies reporting effective absorption of vitamin B6 by the skin.[16]

Discussion

The clinical diagnosis for this patient was nontropical sprue; necropsy supported this opinion and in addition revealed extensive involvement of the lymphatic system with reticulum cell sarcoma. The latter condition could satisfactorily explain the terminal severity of the malabsorption if intestinal lymph nodes were affected; this seemed to be the case by macroscopic exami-

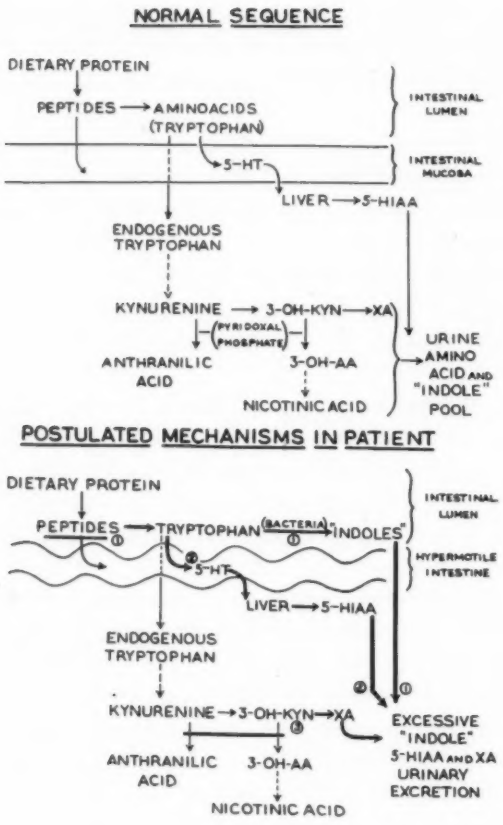

Fig. 5. Suspected deviations of tryptophan metabolism in patient: intraluminal gut flora effect, 5-HT "shunt" and hypermotility effect, and vitamin B_6 deficiency effect.

nation of mesenteric nodes. It is unfortunate that microscopic examination was not performed to verify the fact.

Regardless of the precision of the pathologic diagnosis, it seems clear that anatomic changes in the small intestine were responsible for the malabsorption syndrome. Diarrhea was part of the syndrome in this patient and the abnormalities of trypophan metabolism seemed to be correlated with the severity of small bowel dysfunction. The point is made because other papers[1, 4] discussing the prominence of 5-HIAA excretion in sprue in particular have drawn attention to its absence in other diarrheal disorders; however, the patients with normal 5-HIAA excretion in these reports appear to have had diseases primarily affecting *large* bowel function. The distinction seems important because there may be a tendency in reading the literature to implicate serotonin (5-HT) metabolism with the primary pathogenesis of the malabsorption syndrome. The thesis of my communication is that all three disorders of tryptophan metabolism described in the patient were phenomena secondary to the malabsorption syndrome.

The heavy generalized urinary indole excretion (represented by excessive IAA excretion in other studies[2, 5]) is a nonspecific finding. Coexistent alterations in gut bacterial flora and increased intraluminal amounts of protein and peptides[17] probably occurred in the patient, both contributing to an increase in intraluminal tryptophan and its metabolites. Suppression by neomycin of intestinal bacteria suppressed the "indoluria." Similar observations (Table I) have been made in Hartnup disease[18, 19] where a genetically determined disorder of amino acid transcellular transport affects tryptophan (as well as other amino acids) with secondary accumulation of intraluminal tryptophan. It seems apparent that heavy urinary indole excretion in sprue is a nonspecific secondary phenomenon reflecting alteration of intestinal transport and metabolism of proteins and peptides.

The disorder of tryptophan metabolism affecting kynureninase activity could reflect protein malnutrition[7] or vitamin B6 coenzyme deficiency[6]; the specific response to pyridoxine with correction of the kynureninase inhibition, favors vitamin B6 coenzyme deficiency. The pathogenesis of this deficiency was not evaluated. However, it was observed that riboflavin given orally to the patient was poorly excreted in the urine; only when given parenterally was there an increased urinary riboflavin excretion and associated improvement in the riboflavin-deficiency dermatitis. Intestinal absorption of riboflavin therefore seemed to be inadequate. A similar fate may have affected the vitamin B6 group. Further studies of the mechanisms and of the effects of vitamin B6 deficiency in the malabsorption syndromes would be of interest.

Alteration of serotonin-5-HIAA metabolism has received the most attention in malabsorption syndromes.[1-4] This abnormality was also observed in the present study. Two new observations have been made concerning the possible reasons for excessive 5-HIAA excretion.

First, excessive excretion was obvious only when intestinal hypermotility was present. This would correlate with the effect of a gluten-free diet on excessive 5-HIAA excretion in sprue; the diet if attended by clinical improvement would correct any diarrhea and hypermotility. Furthermore, Adams[20] has reported that a correlation exists between small bowel hypermotility and elevated serum 5-HT levels in normal subjects. The correlation between intestinal hypermotility and urinary 5-HIAA excretion appears to have some justification.

Second, the excessive 5-HIAA excretion seemed to reflect an increased amount of tryptophan metabolized along the 5-HT pathway. The major site for serotonin production is the gastrointestinal mucosa[9]; 5-HT is then converted to 5-HIAA in the liver and excreted from the blood, by active transcellular transport, into renal tubular urine.[10] The apparent though unproved observation that intestinal hypermotility can augment the rate of 5-HT and 5-HIAA metabolism encourages renewed consideration of the effect of intestinal motility on argentaffin cell apoenzyme activity and on transcellular transport of tryptophan.

A schematic representation of the probable pathogenesis of these abnormali-

ties of tryptophan metabolism and their relation to alteration in urinary indole excretion is presented in Fig. 5.

Certain other considerations arise from the studies in this patient. Intestinal bacterial metabolism of tryptophan can result in formation of IAA and tryptamine.[5] The latter in particular has neurotoxicity.[13] These factors should be considered in the clinical assessment, although no evidence of tryptamine toxicity was observed in this subject. An endogenous shunt in tryptophan, in combination with deficiencies of vitamins themselves regulating tryptophan metabolism, could impair nicotinamide formation leading to further metabolic disturbance. These considerations emphasize that malabsorption syndromes should not be considered solely in terms of their effect on dietary fat, protein, and carbohydrate absorption.

The facilities for these studies were generously provided by Prof. C. E. Dent. Miss Diana Sykes supervised the diet and Dr. E. N. Rowlands and Prof. Dent were most helpful in discussing the general problem.

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Significance of alterations in extraction and distribution of colloidal chromic phosphate in patients with liver disease

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Measurements of the efficiency of extraction of P^{32}-labeled chromic phosphate by the liver in 15 patients showed a decreased efficiency related to the severity of hepatic dysfunction. Results for hepatic blood flow uncorrected for extraction were similar to those of other workers but when corrected for extraction efficiency appeared too large. These corrected values suggested significant extrasplanchnic removal of the colloid. Tissue distribution of the colloid was studied in 8 patients in whom the chromic phosphate was injected intravenously from 8 hours to 19 days before death. In 3 patients without significant liver disease 82 to 100 per cent of the administered dose was recovered from the liver and spleen; in 5 patients with cirrhosis the amount recovered from these organs was from 26 to 56 per cent and the bone marrow was shown to be the major alternative site of colloid deposition. In 2 of these cirrhotic patients, it was inferred that the rate of colloid removal by the marrow was of the same order as that by the liver and spleen.

These findings invalidate methods using colloidal chromic phosphate for the measurement of hepatic blood flow in the presence of impaired liver function. There is a large increase in the phagocytic activity of the bone marrow, relative to that of the liver and spleen, in cirrhosis of the liver when subcritical doses of colloidal chromic phosphate are injected intravenously.

Measurement of the rate of disappearance from the bloodstream of intravenously injected colloidal particles such as those of P^{32}-labeled chromic phosphate,[1-3] radiogold,[4-7] and radioiodinated denatured serum albumin[8, 9] has been considered to be a satisfactory method for estimating hepatic blood flow.

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Following our demonstration that colloidal radiogold was unsatisfactory for this use because of a low and variable efficiency of extraction by the liver, with evidence of extrasplanchnic removal in some patients with severe liver disease,[10] it was decided that other apparently suitable colloids should be studied further to determine their behavior, especially in the presence of hepatic insufficiency. With this in view we have investigated the rate of disappearance from the bloodstream, the efficiency of hepatic extraction, and the sites of removal of P^{32}-labeled chromic phosphate in the presence and absence of liver disease.

Methods

Subjects. Measurements of hepatic extraction were performed on one patient with a duodenal ulcer and on 14 others suffering from hepatic diseases of variable severity; in all of the patients the diagnosis was confirmed by percutaneous liver biopsy. In 6 of this latter group biochemical evidence of hepatic insufficiency was minimal.

Tissue distribution of the radioactive colloid, injected intravenously during life, was measured after death in 8 cases. In one of these patients there was no evidence of liver disease either during life or at autopsy; in another 2 there were hepatic diseases which did not contribute to the immediate cause of death in either case; and there were 5 patients who died of hepatic failure due to cirrhosis of the liver and its complications. At the time of injection of the colloid no patient was in shock or had been given any drug known to affect hepatic blood flow. Of the cirrhotic patients 3 were in hepatic coma when the colloid was administered, the fourth subsequently bled from acute gastric erosions and died in hepatic coma, and the fifth, who had been bleeding from esophageal varices, died 19 days later after a thoracotomy for ligation of varices complicated by pulmonary collapse.

*Colloidal P^{32}-labeled chromic phosphate.** The P^{32}-labeled chromic phosphate used in this study was suspended in isotonic saline as a colloid having an average particle size of 1 to 2 μ. Its suitability for measuring the hepatic blood flow was confirmed by the manufacturer by studying its behavior in normal dogs and mice.[11]

Measurement of hepatic extraction of the radiocolloid. After the intravenous injection of approximately 10 μc of the radiocolloid, samples of peripheral arterial and hepatic venous blood were obtained at 2, 3, 4, 5, 6, 7, and 10 minutes using the same conditions and technique as described previously.[10] The radioactivity of each sample was recorded, the counts per minute were plotted on semilogarithmic paper as a function of time, and the lines of best fit drawn through the points for peripheral artery and hepatic vein levels.

The efficiency of extraction of the colloid was calculated at minute intervals from 2 to 10 minutes as the difference between the radioactivity of simultaneously obtained samples of peripheral arterial and hepatic venous blood expressed as a percentage of the former.

In each subject the estimated hepatic blood flow (EHBF [uncorrected]) was calculated by the usually accepted method which assumes the absence of significant extrasplanchnic removal of the colloid and a hepatic efficiency of extraction of 100 per cent so that EHBF (uncorrected) $= k \times BV$ where the disappearance constant (k) is related to the half-time ($T_{1/2}$) of disappearance of the colloid by the expression $k = \frac{0.693}{T_{1/2}}$. Blood volume (BV) was assumed to equal 70 ml. per kilogram.

An additional value was derived for estimated hepatic blood flow corrected for extraction efficiency (EHBF [corrected]) where

$$\text{EHBF (corrected)} = \frac{\text{EHBF (uncorrected)} \times 100}{E},$$

E being the mean efficiency of extraction over the period from 2 to 10 minutes.

*Supplied by Dr. E. Dobson, Donner Laboratory, University of California, Berkeley, Calif.

Table I. Results of hepatic extraction of colloidal P^{32}-labeled chromic phosphate and estimated hepatic blood flow

Patient	Diagnosis	Sex	Age	Bromsulphalein retention (% at 45 minutes)	$T\frac{1}{2}$ (minute)	k (per minute)	Hepatic extraction of colloidal chromic phosphate* (%)				EHBF (uncorrected)† (ml. per minute)	EHBF (corrected)‡ (ml. per minute)
							2 minutes	6 minutes	10 minutes	Mean 2-10 minutes		
R. M.	Fatty liver	M	58	4	2.7	0.26	96	89	71	87	1,100	1,300
F. G.	Fatty liver	M	45	2	3.0	0.23	92	88	83	87	1,200	1,400
A. B.	Duodenal ulcer	M	57	5	2.0	0.35	95	89	70	86	1,800	2,100
W. M.	Cirrhosis	M	42	11	2.6	0.27	88	79	67	79	1,400	1,800
A. L.	Cirrhosis	M	55	16	3.1	0.22	53	53	53	53	1,100	2,100
D. R.	Cirrhosis	M	38	12	2.4	0.29	66	67	46	61	1,300	2,100
E. J.	Hemochromatosis	M	57	3	1.6	0.43	92	85	35	76	1,700	2,200
T. M.	Cirrhosis	M	63	11	2.7	0.26	75	70	63	70	1,100	1,600
N. O.	Fatty liver	F	46	3	1.8	0.39	93	84	64	82	1,400	1,700
S. G.	Cirrhosis	M	33	19	2.8	0.25	51	46	42	46	1,200	2,600
V. M.	Cirrhosis	M	43	20	3.2	0.22	64	55	47	55	1,000	1,800
A. C.	Cirrhosis	M	51	19	2.2	0.31	65	62	60	62	1,600	2,600
A. A.	Cirrhosis	M	40	5	3.3	0.21	87	87	84	87	1,300	1,500
S. S.	Cirrhosis	F	31	8	1.5	0.46	77	69	61	70	1,800	2,600
K. R.	Hemochromatosis	M	43	3	2.1	0.33	93	96	89	93	1,700	1,800

*Result for extraction at 3, 4, 5, 7, 8, and 9 minutes were calculated but not included in the table.
†Estimated hepatic blood flow using the disappearance of chromic phosphate, uncorrected for inefficient extraction.
‡EHBF Uncorrected $\times \frac{100}{\text{Mean efficiency of extraction}}$.

Tissue distribution of P^{32}-labeled chromic phosphate. Ten to twenty microcuries of radiocolloid was injected intravenously into each of 12 patients with potentially fatal diseases. In two the rate of disappearance of the colloid was measured, and autopsies were performed on 8 who died at intervals of from 8 hours to 19 days after injection. The liver and spleen of each of these 8 patients were separately progressively homogenized with water in a Waring Blendor. In 3 patients a mixture of marrow and cancellous bone from the lumbothoracic vertebrae was weighed and ground and the marrow extracted by repeated washings with water in a Waring Blendor. The bone residue was dried between 70 and 80° C. and the weight of the extracted marrow considered to be the difference between the original weight of marrow and bone mixture and the dry weight of the bone residue.

The radioactivity of aliquots of each specimen was measured and compared with a standard solution. The results of colloid distribution to the liver and spleen were expressed as a percentage of the administered dose of radioactivity. In the cases in which marrow radioactivity was measured, the weight of active marrow necessary to account for the radioactivity not present in liver and spleen was calculated.

Results

Hepatic efficiency of extraction of colloidal chromic phosphate. The mean values of hepatic extraction over the period from 2 to 10 minutes were from 46 to 93 per cent. Values of T½ varied from 1.5 to 3.3 minutes, these results giving disappearance constants from 0.21 to 0.46 per minute (Table I).

EHBF (uncorrected) ranged from 1,000 to 1,800 ml. per minute and EHBF (corrected) from 1,300 to 2,600 ml. per minute (Table I).

Tissue distribution of the colloid. In one patient with a normal liver and another with acute myeloid leukemia, 91 and 100 per cent, respectively, of the injected dose of colloid were found in the liver and spleen (Table II). In a patient with metastatic carcinoma of the liver 82 per cent was found in these organs (BSP retention of 20 per cent at 45 minutes). In 5 patients who died of cirrhosis the total hepatic and splenic contents of chromic phosphate varied from 26 to 56 per cent. In 3 of these patients, the concentration of marrow radioactivity was measured and it was assumed that the colloid, not accounted for in the liver and spleen, was in the bone marrow. The resultant calculated weights for active bone marrow were 819, 1,704, and 1,171 Gm. (Patients E. S., W. D., and E. C., respectively). These values lie within the normally accepted range for active marrow weight.[12]

Values for T½ in 2 of these patients who died of cirrhosis were 2.6 and 3.1 minutes, the former result being in a patient in whom 60 per cent of the colloid was calculated to be present in an active marrow that weighed 1,171 Gm.

Discussion

Two criteria must be satisfied in all circumstances if a colloid is to be suitable for measuring hepatic blood flow: it should be removed completely by the reticuloendothelial cells of the liver on a single passage through that organ and there should be no significant extrasplanchnic extraction.[1, 8] If only the second of these requirements is fulfilled the method is still valid provided hepatic extraction is determined in each case and remains relatively con-

Table II. Tissue distribution of colloidal P^{32}-labeled chromic phosphate

Patient	*Diagnosis*	*Sex*	*Age*	*Time interval between injection of chromic phosphate and death*	*T½ (minute)*	*P^{32}-labeled chromic phosphate recovered at autopsy (% of administered dose)*			*Calculated weight of active marrow† (Gm.)*
						Liver	*Spleen*	*Bone marrow**	
F. M.	Cerebral hemorrhage	F	69	17 days	—	78	13	—	—
L. D.	Acute leukemia	M	28	3 days	—	100	5	—	—
P. T.	Metastatic carcinoma	F	34	5 days	—	65	17	—	—
M. L.	Cirrhosis	F	39	34 hours	—	13	13	—	—
J. M.	Cirrhosis	M	53	8 hours	3.1	20	32	—	—
E. S.	Cirrhosis	M	62	4 days	—	25	31	44	819
W. D.	Cirrhosis	M	56	19 days	—	26	25	49	1,704
E. C.	Cirrhosis	M	47	3 days	2.6	27	13	60	1,171

*Bone marrow content assumed to be that unaccounted for in liver and spleen.

†Weight of active marrow $= \frac{100 - (\text{percentage of administered dose in liver and spleen})}{\text{Percentage of administered dose in 1 Gm. marrow}}$.

stant throughout the period of observation. Both of these requirements have been shown to be satisfied when P^{32}-labeled chromic phosphate is used for measuring liver blood flow in normal animals.[1]

The diseased liver cannot be assumed to extract this colloid with the same efficiency as the normal liver, as our results show that the mean efficiencies of hepatic extraction of chromic phosphate in a group of 15 subjects varied from 46 to 93 per cent. There would be, therefore, major inaccuracies in estimating hepatic blood flow based on the rate of disappearance of the colloid from peripheral blood if no correction was made for inefficient extraction. Such errors would be greater in patients with poor liver function, since low efficiencies of extraction were always demonstrated in this group.

In addition, however, it has been shown that the bone marrow was a very significant site of removal of the colloid in patients with severe liver disease. In 5 cirrhotic patients, the liver and spleen together removed only from 26 to 56 per cent of the administered dose of the colloid, whereas in 3 patients without serious hepatic disease, these organs contained from 82 to 100 per cent. In 3 of this former group, if it was assumed that the remainder of the dose of radioactivity was present in the bone marrow, calculated weights of this tissue lie within the normal range. Also, in 2 of these cirrhotic patients, the rate of disappearance of the colloid was found to be within the range of results obtained in subjects with good hepatic function, indicating that the rate of colloid removal by the marrow was of the same order as that of the liver and spleen. The presence of significant extrasplanchnic removal of the colloid was also suggested by our results for EHBF (corrected) (Table I) which were higher than those described in liver disease when constant infusion techniques were used.[13-16]

We do not consider that our results were due to unsatisfactory colloid preparation because in our patients with good hepatic function both criteria necessary for a colloid to be suitable for measurement of hepatic blood flow were satisfied. It is theoretically possible that in our subjects with severe liver disease the colloid was extracted initially by the liver and spleen and that a portion was later released into the circulation and then preferentially removed by the bone marrow; however, inert colloids of all types have been demonstrated to remain permanently at the sites of initial deposition,[17] except after thermal injury.[18]

We consider our results of tissue distribution of the colloid in patients with poor hepatic function are best explained by a combination of reduced efficiency of the hepatic removal mechanism and increased bone marrow activity. One or a combination of the following: an increase in active marrow blood supply, size, or efficiency of colloid extraction theoretically could be responsible for this increase in marrow phagocytic activity in cirrhosis. A hyperplastic marrow has been demonstrated in patients with this disease[19] although it cannot be assumed that such a change is associated with an increase in marrow phagocytic activity.

It is concluded first that although the rate of disappearance from the peripheral blood of an injected dose of P^{32}-labeled chromic phosphate can be used to measure blood flow through normal livers, the method is invalid when there is abnormal liver function. Secondly, there is an increase in the phagocytic activity of the bone marrow, relative to that of the liver and spleen, in cirrhosis of the liver as measured by the tissue distribution of colloidal P^{32}-labeled chromic phosphate given in subcritical doses. Under these circumstances the rate of clearance is considered to be blood flow dependent and not related to the dose size or over-all activity of the reticuloendothelial system.

We wish to thank Prof. C. R. B. Blackburn, Professor of Medicine in the University of Sydney and Director of the Clinical Research Unit, Royal Prince Alfred Hospital, for his encouragement and advice, the Honorary Medical Officers who permitted these studies to be performed on patients under their care, and the assistance of the Director and staff of the Hallstrom Institute of Cardiology, Sister S. Ferguson and Miss A. Carolan.

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Glucosamine and acetylglucosamine tolerance in man

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and RICHARD J. WINZLER, PH.D. *Chicago, Ill.*

Blood levels of glucosamine and of N-acetylglucosamine have been determined following intravenous administration of 12 to 20 Gm. of the two amino sugars. Glucosamine is somewhat more rapidly removed from the blood stream than is acetylglucosamine; the half life of glucosamine in the blood stream is 150 minutes and that of acetylglucosamine is 220 minutes. Administration of insulin has no effect on disappearance from blood of acetylglucosamine, but may slightly increase the removal of glucosamine. About one half of the glucosamine and of the acetylglucosamine was excreted unchanged in the urine within 12 hours. The metabolic fate of the remainder, which was not excreted, remains unknown.

Glucosamine is an important constituent of many glycoproteins and mucopolysaccharides of serum and tissues.[1, 2] Much recent work has been concerned with pathways of glucosamine biosynthesis from glucose.[3-6] However, relatively little work has been done on the catabolism of exogenous or endogenous glucosamine or N-acetylglucosamine, or on the utilization of these compounds in animals. Glucosamine metabolism in man has been studied by Weiden and Wood,[7] who have also summarized much of the early work.

This paper represents an extension of their work. Glucosamine and acetylglucosamine metabolism in human beings has been studied by determining tolerance curves with intravenously administered glucosamine and N-acetylglucosamine. The influence of insulin administration on the tolerance curves was studied and blood glucose levels were followed in a few patients with diabetes.

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Materials and methods

Some of the patients at the start of the project were cancer patients in a terminal condition but most were patients with normal liver function and presumed normal carbohydrate metabolism. No consistent differences in any of these were apparent.

Four different types of experiments were carried out. The first was intravenous administration of about 225 mg. per kilogram body weight of glucosamine hydrochloride prepared by filtration through a sintered glass ultra filter under sterile conditions using pyrogen-free water. The second experiment was intravenous administration of 225 mg. per kilogram body weight glucosamine hydrochloride followed by 7 units of insulin (regular) intravenously 5 to 10 minutes after completion of glucosamine administration.

The third and fourth types of experiments were similar to the first and second, respectively, except that 20 Gm. of N-acetylglucosamine was administered in place of the glucosamine hydrochloride.

Blood samples were drawn at intervals and the proteins precipitated by the Somogyi method.[8] Glucose was determined by a glucose oxidase method on certain patients. Free glucosamine was determined in the protein-free filtrates by the method of Neuhaus and Letzering.[8] Free N-acetylglucosamine was determined without hydrolysis by the method of Morgan and Elson.[9] Urine samples were collected from one normal subject before and for 24 hours after administration of 20 Gm. glucosamine hydrochloride, of N-acetylglucosamine, and of glucosamine, and 7 units of insulin. Analysis for the appropriate amino sugar was performed on a 1:1,000 dilution of each urine sample, under which conditions interference from normal urine components was insignificant.

The amino sugars were administered through the antecubital veins as a 20 per cent solution over a 10 minute interval. There was no evidence of unfavorable reaction in any of the subjects.

Results

The blood levels of glucosamine and of acetylglucosamine in the four groups of experiments are shown in Fig. 1, *A*, *B*, *C*, and *D*. In these curves the amino sugar levels are expressed as per cents of the 15 minute levels. The logarithm of the average concentrations is plotted against time in Fig.

Table I. Cumulative excretion of glucosamine and acetylglucosamine in the urine

Hours	*20 Gm. glucosamine HCl (Gm.)*	*20 Gm. acetylglucosamine (Gm.)*	*20 Gm. glucosamine and 7 units insulin (Gm.)*
0	0	0	0
1	4.50	4.72	4.91
2	6.90	7.20	6.84
3	8.60	9.85	8.18
5	—	10.60	—
6	11.0	—	—
8	—	—	9.84
11	—	11.71	—
16	11.44	—	—
18		10.71	10.22
24	11.44	10.71	10.22

A normal subject (62 kilograms) was fasted overnight and 20 Gm. of glucosamine hydrochloride or of acetylglucosamine administered intravenously. In the third experiment 7 units of regular insulin was given intravenously immediately. Urine samples were collected as indicated and analyzed for the administered sugar.

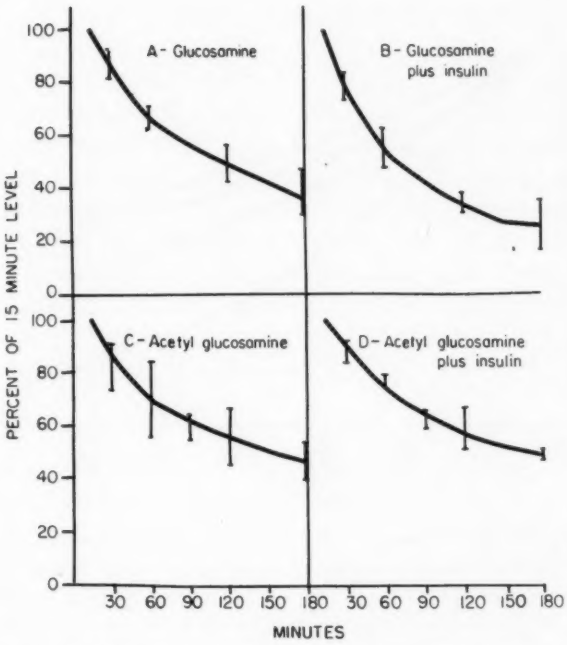

Fig. 1. Blood levels of amino sugars following intravenous administration of glucosamine hydrochloride or acetylglucosamine. Values expressed as per cent of level 15 minutes after completion of intravenous administration. Standard deviations are shown by the bars. *A*, glucosamine hydrochloride (225 mg. per kilogram), 5 subjects; 15 minute level 88.1 ± 10.4 mg. per cent. *B*, glucosamine hydrochloride (225 mg. per kilogram) plus insulin (7 units), 5 subjects; 15 minute level 78.2 ± 8.9 mg. per cent. *C*, N-acetylglucosamine (20 Gm. per subject), 5 subjects; 15 minute acetylglucosamine level 110.8 ± 7.1 mg. per cent. *D*, N-acetylglucosamine (20 Gm. per subject), 4 subjects; 15 minute acetylglucosamine level 128 ± 3.6 mg. per cent.

2 to permit calculation of half life of the administered amino sugars. The half life for glucosamine is somewhat slower than that calculated from the data of Weiden and Wood.[7]

It would appear from Figs. 1 and 2 that glucosamine disappears from the blood stream somewhat more rapidly than does acetylglucosamine, the average half lives, calculated for the disappearance during the third hour, being 150 and 220 minutes, respectively.

Administration of 7 units of insulin had no obvious effect on the disappearance rate of the acetylglucosamine, but appeared to have an accelerating effect on glucosamine removal from the blood in the first 2 hours.

The removal of the amino sugars from the blood represents the sum of catabolic oxidation of the compounds, the utilization of the compounds or their metabolic fragments for synthetic reactions, and loss by excretion in the gut or urine. In an effort to evaluate the extent of utilization of the amino sugars in metabolic reactions, the 24 hour urinary excretion of the unaltered sugars was determined in a normal individual given sequentially glucosamine, acetylglu-

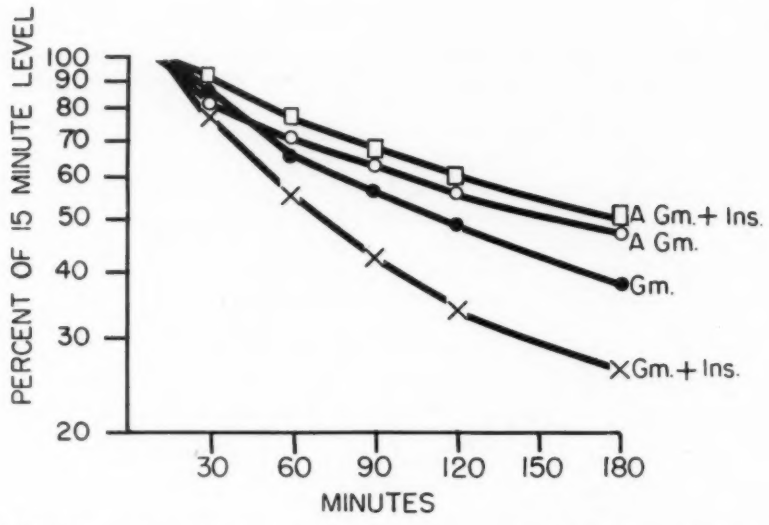

Fig. 2. Half life of administered amino sugars. Average levels of glucosamine or of acetylglucosamine (expressed as per cent of 15 minute levels). Half lives calculated from the slope during the final hour are: glucosamine, 150 minutes; glucosamine plus insulin, 150 minutes; acetylglucosamine, 220 minutes; and acetylglucosamine plus insulin, 220 minutes.

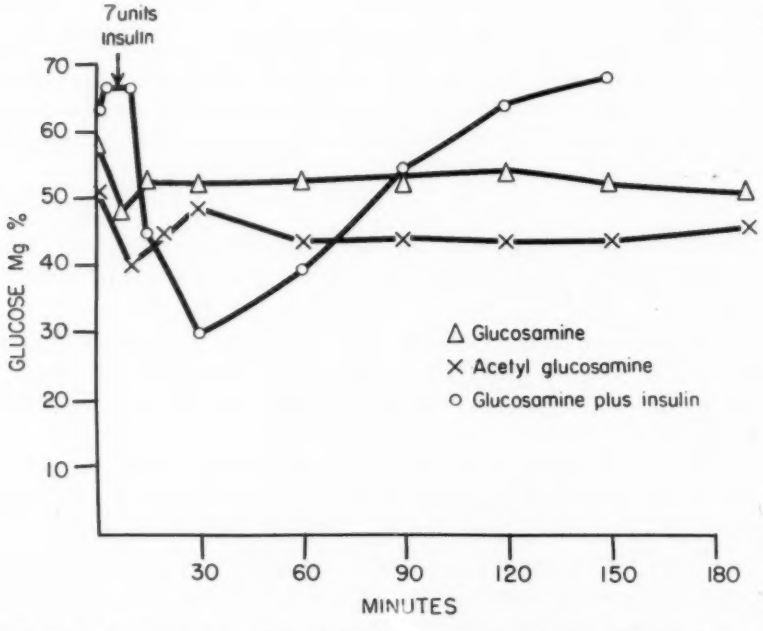

Fig. 3. Effect of glucosamine and N-acetylglucosamine on blood sugar levels in a normal subject. Twenty grams of the amino sugars was administered intravenously to a fasted normal individual (62 kilograms) over a 10 minute interval. Two weeks elapsed between successive experiments.

cosamine, and glucosamine plus insulin. The cumulative urinary excretion are shown in Table I.

It is evident that somewhat over 50 per cent of the amino sugars was excreted unaltered under the conditions of the tolerance test described.

One of the questions with which we were concerned was that raised by the work of Spiro[10] who found that the in vitro oxidation of glucose by liver slices could be inhibited by glucosamine. In the experiments shown in Fig. 3 it was evident that administration of 20 Gm. of glucosamine HCl or of acetylglucosamine to a normal individual had little influence on blood glucose levels or on the effect of insulin on blood glucose levels. Although not conclusive, these data suggest that glucose utilization is not hampered by the amino sugars in the amounts employed.

Discussion

It is evident from Table I that about half of the administered amino sugars are excreted unchanged in the urine under the conditions employed in this work. The urine excretion fell to very low levels by the end of 12 hours. However, a large portion, approximating one half of that administered, cannot be demonstrated in the urine and it seems likely that this is catabolized to CO_2 or is incorporated into tissue components. These results confirm and extend the observations of Weiden and Wood[7] who showed that excretion of intravenous glucosamine occurred within 24 hours and that only about one half was excreted. Since their urine samples were hydrolyzed the possibility of missing a conjugated derivative was avoided.

It would seem likely that a much larger proportion of the amino sugars would have been metabolized had they been administered at much lower levels. Tracer studies with labeled amino sugars have not yet been carried out in man. In the rat, however, about 25 per cent of a tracer dose of acetylglucosamine and 5 per cent of a tracer dose of glucosamine were oxidized to CO_2 in a 4 hour interval.[11]

It appears possible that insulin administration might have had a very slight stimulating effect on the removal of glucosamine from the circulating blood, although this effect may well have been an indirect one. Acetylglucosamine disappearance was not significantly affected by insulin. Weiden and Wood[7] found that the disappearance of glucosamine from the blood was not different from normal in patients with diabetes.

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Abstracts

68. The creatine phosphokinase activity of normal and arteriosclerotic human aortic tissue

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The activity of creatine phosphokinase (adenosine triphosphate-creatine transphosphorylase) was determined on 90 aortic samples (thoracic descending) obtained fresh at autopsy from individuals ranging in age from 1 month to 86 years. The activity assays were performed under zero order conditions by a modification of the procedure of Chappell and Perry, in which phosphate is transferred enzymatically from creatine phosphate to ADP and the free creatine thus liberated is measured colorimetrically. The Eggleton method was used for the spectrophotometric determination of this compound. A complete reagent blank and a tissue blank containing creatine phosphate but no ADP were run with each tissue sample. For comparative purposes the activity assays were carried out separately on normal and arteriosclerotic tissue portions of the same blood vessels.

The average creatine phosphokinase activity observed for normal human aortic tissue was 0.095 mM. of creatine liberated per gram wet tissue per hour. The enzyme activity showed a definite tendency to decrease with age, the coefficient of correlation between age and creatine phosphokinase activity being -0.78 ($t = 11.5$). The mean activity of arteriosclerotic tissue portions was only 54 per cent of that exhibited by the normal tissue of the same arterial samples (t of difference $= 6.80$).

Because creatine phosphokinase is linked to processes producing and utilizing energy, the finding of a rather high activity of this enzyme in normal human aortic tissue and of consistently lower values in arteriosclerotic tissue areas may represent observations of definite metabolic significance.

69. The effect of extracorporeal circulation upon the tissue digoxin concentration in the dog

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(By Invitation) *St. Louis, Mo.*

The effect of total body perfusion with extracorporeal support upon the concentration of digitalis glycosides in tissues is not known. Therefore, the regulation of dosage in patients in the immediate postoperative period is very difficult.

Experiments were devised using 4 dogs which were digitalized with tritium-labeled digoxin (1 mg. digoxin was labeled with 50.9 μc of tritium) 75 to 180 minutes prior to cardiopulmonary bypass with a Kay-Anderson heart-lung pump. During the 30 minute perfusion, blood samples were obtained every 5 minutes from the venous line of the pump (mixed venous blood of the dog). Arterial blood samples were obtained at 10 minute intervals (pooled blood of the heart-lung apparatus).

The animals were sacrificed and samples of heart, kidney, and liver were obtained.

The samples of blood and tissue were analyzed for radioactivity using a Packard Tri-Carb liquid scintillation spectrometer with a 2 channel pulse height analyzer.

Pieces of atrial muscle were obtained before and after bypass to evaluate the pattern of contractility for digitalis effect.

No radioactivity could be detected in the perfusion fluid of the heart-lung apparatus during bypass and none could be detected in the blood reservoir after perfusion.

The average concentration (3 dogs) of tritium-labeled digoxin in the heart muscle was 13.4 mμc per gram of tissue, in the kidney it was 57.4 mμc per gram of tissue, and in the liver it was 5.96 mμc per gram of tissue. These figures represented, respectively, 1.86, 8.2, and 3.65 per cent of the total administered tritium-labeled digoxin. These values were in the same range as those of the control animal, which was digitalized and did not undergo total body perfusion.

The pattern of atrial muscle contractility did not show a diminished degree of digitalization after perfusion.

This pilot experiment suggests that total body perfusion does not appreciably affect the digoxin content of the heart, liver, and kidneys in the dog. Experiments are in progress to determine if this conclusion applies to man.

70. Immunohistochemical studies of experimental colitis

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Chicago, Ill. Introduced by Walter L. Palmer, M.D.

An experimental "colitis" in animals may be produced by a variety of alleged hypersensitivity reactions. The present work has investigated some immunohistochemical aspects of the Auer approach, whereby an immunized animal has been considered to localize subsequent amounts of the intravenously injected antigen at a site of irritation or inflammation, with a resultant local hypersensitivity response.

Sensitized rabbits were subjected to mild, nonspecific colon irritation (dilute formalin enemas) over a 2 week period while receiving additional intravenous injections of specific antigen (crystalline egg albumen, [CEA]). Earlier studies in our laboratory had revealed hemorrhagic ulceration in the colons of 15 of 17 animals subjected to this modification of the Auer reaction. In an additional group of 33 rabbits, we have now demonstrated gross and microscopic changes, including edema, hemorrhage, ulceration, perivascular round cell infiltration, and mucosal and/or submucosal infiltrations of mononuclear cells and eosinophils. Comparable reactions have not been observed in appropriate control animals.

Gamma globulin from the pooled sera of CEA-sensitized rabbits was conjugated with fluorescein isothiocyanate. The fluorescent antibody technique of Coons and Kaplan was then successfully employed to demonstrate specific fluorescent staining of antigen at the sites of the inflammatory colon reactions, but not in normal, uninvolved colonic areas of these animals or in the colons of sensitized and nonsensitized control rabbits. This antigen often was seen in perivascular areas, or it was identified in close proximity to the vascular endothelium or in association with the glandular cells of the colonic mucosa. This work provides, for the first time, objective evidence for the sites of localization of antigen in tissues of the Auer reaction.

71. The effect of aging on the desoxyribonuclease system, body and organ weight, and cellular content

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Investigation on the changes with aging in the desoxyribonuclease system in the organs and serum of mice has been carried out. We have observed a five- to sixfold increase in the acid DNase activity of the thymus and one of three- to fourfold in the spleen during maturation. The activity remains stable thereafter. The liver and kidney revealed slight progressive increase with age in acid DNase activity, while the bone marrow showed a fall with aging.

Alkaline DNase activity was found to rise approximately threefold in the liver and approximately sevenfold in the kidney during maturation. In maturity and senescence the levels tended to fall slightly. The activity of the inhibitor of serum desoxyribonuclease fell with age in all the organs investigated. Observations on DNA content per gram of tissue and per isolated nucleus failed to reveal any significant changes during growth, indicating no marked changes in polyploidy or in cell mass. Organ growth in kidney, liver, and spleen appears to be due to increase in number of cells. The activity of alkaline DNase per average cell was similar in liver and kidney. In the kidney, the alkaline DNase activity was approximately one third to one half of the acid DNase activity, while in the liver the alkaline DNase activity was only one tenth of the acid DNase activity. Thymocytes had the lowest acid DNase activity per cell of the several tissues analyzed. The tendency of the nuclease activities to rise with age and of the inhibitor activity to fall suggests that the enzyme system may have a primarily catabolic role. The rapid changes in spleen and thymus acid DNase activity during the first 100 days of life, when body growth is also maximal, suggests possible hormonal dependence.

72. A syndrome clinically resembling adrenal cortical insufficiency associated with long-term busulfan (Myleran) therapy

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Boston, Mass. Introduced by Albert B. Hagedorn, M.D.

This paper describes a syndrome appearing in patients with chronic granulocytic leukemia who were treated with busulfan (Myleran) for protracted periods. The syndrome is characterized by hyperpigmentation of the skin, severe weakness, fatigue, anorexia, nausea, and loss of weight.

The complete syndrome developed after treatment with busulfan for 1 to 5 years, the total dose having varied from 820 to 5,710 mg. The status of the leukemia did not appear to play a significant role in the syndrome, because the latter developed during hematologic remission. Fatigue and weakness were pronounced; anorexia and loss of weight of as much as 50 pounds were constant features. Nausea was present in 3 patients, and diarrhea was found in 2. Generalized brownish hyperpigmentation of the skin was noted which was most pronounced on the trunk, face, and hands; the mucous membranes and palmar creases were not hyperpigmented. Biopsy of the skin revealed pigment granules which showed a strongly positive dopa reaction but did not stain positively for iron. A split section of the skin showed no increase in the number of melanocytes. Generalized hyperpigmentation of the skin has been reported in only 4 cases of chronic granulocytic leukemia in which busulfan was not given. The clinical picture simulated Addison's disease, but this diagnosis was excluded because of the finding of normal urinary excretion of 17-hydroxycorticosteroids and 17-ketosteroids before and after ACTH stimulation.

The mechanism of the production of this syndrome is obscure. Busulfan was applied locally to the arms of the authors for 4 weeks without evidence of local hyperpigmentation.

The treatment of the syndrome is unsatisfactory.

73. Report of familial occurrence of dermatomyositis and a family survey

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In only a few rare instances has the familial occurrence of dermatomyositis been observed. During the past three years a clinical diagnosis of dermatomyositis has been made in 2 first

cousins admitted to our hospital. The patients were girls, aged 13 and 17 years. Diagnosis in both cases was made on the basis of history, physical findings, and laboratory studies. Definite histologic changes were present in the muscle biopsy of one patient. The other patient had a normal muscle biopsy.

The availability of the other members of both families provided a unique opportunity for a family survey. Blood samples from all of the 25 living immediate relatives of both patients were obtained; 23 relatives were examined for evidence of connective tissue disease.

Markedly elevated serum gamma globulins were noted in 4 relatives. Four other relatives had marginally elevated serum gamma globulins. Five of the eight elevated serum gamma globulins were present in asymptomatic siblings of the 2 patients. L.E. preparations were all negative except in one instance where numerous atypical L.E. cells and rosettes were present. Latex slide tests were weakly positive in 2 patients, one of whom was asymptomatic. All latex tube tests were negative. Cryoglobulins were negative except for two equivocal tests in asymptomatic individuals.

The mother of one patient had definite changes of rheumatoid arthritis on the basis of physical examination, hand x-rays, and a weakly reactive latex slide test. The grandmother of the other patient had possible rheumatoid arthritis as well as osteoarthritis. She died before hand films could be taken. A third relative also had possible rheumatoid arthritis clinically. Her hand films were negative.

Abnormal serum gamma globulins in asymptomatic relatives of patients with systemic lupus erythematosus and scleroderma have been previously recorded. This is the first report of elevated gamma globulins noted in asymptomatic relatives of patients with dermatomyositis. The finding of 3 immediate family members with evidence of rheumatoid arthritis is also of considerable interest. Although this may be coincidence, the possibility of a meaningful interrelationship among the various connective tissue diseases, genetic or otherwise, deserves careful consideration.

74. Evidence for the existence of new coenzymes derived from thiamine

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Introduced by E. B. Reeve, B.M.

It has been known for some time that a rat on a high fat diet requires less thiamine than one on a high carbohydrate diet. It has also been observed that a rat made deficient in thiamine by the oral administration of oxythiamine develops different deficiency symptoms from one made deficient by pyrithiamine. It has been suggested that this could be explained by the existence of another coenzyme of thiamine besides thiamine pyrophosphate, and that oxythiamine inhibits one coenzyme and pyrithiamine inhibits the other. It seemed possible that the thiamase hydrolytic products of thiamine might act as coenzymes. Accordingly, two groups of animals were fed a suboptimal thiamine diet and given Sulfasuxidine. One group received a supplement of the pyrimidine hydrolytic product of thiamine and the second the thiazole hydrolytic product. The group receiving the thiazole hydrolytic product showed a weight gain comparable with that of the control animals receiving the equivalent amount of pure thiamine, and significantly greater than the control animals on the suboptimal thiamine diet. The group receiving the pyrimidine hydrolytic products showed a less marked weight gain than the former group, but still significantly greater than the group on the suboptimal thiamine diet. In another set of experiments, rats were rendered thiamine deficient by feeding oxythiamine. The normal rate of weight gain was partially restored by feeding the pyrimidine moiety, and to a less extent by feeding the thiazole moiety. Therefore, it appears that both the thiazole and the pyrimidine moieties are active in metabolic processes essential for the health and growth of rats.

75. Acute water loading as a diagnostic test for the inappropriate ADH syndrome

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Inappropriate secretion of antidiuretic hormone (ADH) has been reported in bronchogenic carcinoma and central nervous system disease. An additional six examples of the syndrome have been found by use of a rapid diagnostic test. The test was performed on patients suspected of having the syndrome who had no evidence of the usual causes for hyponatremia. After a control collection of urine and serum, oral tap water or intravenous 5 per cent dextrose in water (20 ml. per kilogram) was given in one hour. Urine and serum specimens were collected hourly for 3 hours and the osmolal, free water, and creatinine clearances and electrolyte excretions were calculated. Patients without hyponatremia served as controls.

Control subjects showed a prompt decrease in urine osmolality (minimum Uosm.: 85 to 184 mOsm. per liter); an increase in free water clearance (maximum C_{H_2O}: +2.1 to +11.2 ml. per minute); and prompt elimination of the water load (52 to 98 per cent within 3 hours).

Six patients with hyponatremia, 2 of whom had cerebral thrombosis, 2 tuberculous meningitis, and 2 bronchogenic carcinoma, responded in a manner suggesting the inappropriate secretion of ADH. The urine remained hypertonic, or became transiently isotonic, to the serum (minimum Uosm.: 255 to 638 mOsm. per liter); significant free water clearance did not appear (maximum C_{H_2O}: −1.0 to +0.1 ml. per minute); and less of the water load was excreted (22 to 69 per cent within 3 hours). The diagnosis of the syndrome was confirmed by balance studies in 4 patients. In 3 patients with unexplained hyponatremia one equivocal and two normal responses occurred.

In 2 patients the test was repeated while diphenylhydantoin HCl, 250 mg., was injected intravenously. Both developed a positive C_{H_2O}. In one patient with tuberculous meningitis a normal response to acute water loading was noted after one month of therapy.

The inappropriate ADH syndrome may be diagnosed in hyponatremic patients by acute water loading, is more frequent than has been recognized, and may be reversed by the administration of diphenylhydantoin or by correction of the associated disease.

76. Antibodies to polioviruses in urine

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Introduced by M. Kenton King, M.D.

Neutralizing substances for polioviruses were recently demonstrated in normal urines and it was shown that certain biologic properties and the kinetics of the neutralization reaction of the urinary concentrates were similar to those of serum containing homologous poliovirus antibody of similar titer (Virology 14: 493, 1961). However, such neutralization was demonstrable only infrequently in urine by the usual tests, and sequential appearance and changes in titers of antibodies in serum and urine remained to be determined.

The sensitivity of the assay for neutralizing antibody could be increased up to fifty to one hundred fold by use of a combination of immunoinactivation (Gard) and the plaque reduction techniques. With this improved method, simultaneous assays of urine and serum for poliomyelitis neutralizing antibodies were done before and after immunization with live-attenuated and formaldehyde-inactivated poliovirus vaccines. When the titers in

the serum by this method were 1:320 or less, urinary antibody was demonstrated in only about one third of the subjects. However, when the titers in serum were higher, antibody usually could also be demonstrated in the urine more than twice as often. Rises in titer of antibody in the urine were generally concordant with those in serum.

The data obtained in this study and those reported by Franklin (J. Clin. Invest. 38: 2159, 1959) using isotopic methods suggest that the urinary antibody (gamma globulin) is derived, at least in part, from corresponding serum fractions.

77. Enterohepatic circulation of bilirubin

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It has not been determined whether bilirubin undergoes an enterohepatic recirculation after excretion into the duodenum. Previous study of this problem has been hampered by lack of a suitable bilirubin label. The problem has now been investigated employing C^{14}-labeled bilirubin.

In preliminary experiments, crystalline unconjugated C^{14}-bilirubin was prepared from rat bile by methods developed in this laboratory. Conjugated C^{14}-bilirubin was prepared by intravenous infusion of unconjugated C^{14}-bilirubin into rats with an external bile fistula. The bile containing conjugated C^{14}-bilirubin was frozen and stored for brief periods until use.

For studying the enterohepatic circulation of bilirubin, rats were anesthetized and provided with an external bile fistula. Bilirubin was instilled into the duodenal lumen through a transoral duodenal tube. Unconjugated C^{14}-bilirubin was dissolved in albumin, bile, or taurocholate, whereas conjugated C^{14}-bilirubin was administered in native bile. Rats were placed in restraining cages permitting separate and quantitative collection of bile, urine, and feces. The excreta were assayed for radioactivity. Bilirubin was crystallized from bile and its specific activity determined.

Following intraduodenal administration of either form of C^{14}-bilirubin, significant absorption and biliary excretion of the radioactive label occurred in all instances. Excretion began during the earliest measured interval, one-half hour, and continued for 15 to 35 hours. Twenty to 40 per cent of the administered radioactivity appeared in the bile during the course of an experiment. Similar quantities of radioactivity appeared in the bile of rats pretreated with neomycin. In contrast, in most animals insignificant amounts of radioactivity were recovered in the urine.

During the initial phase, absorption and excretion were more rapid for unconjugated than for conjugated C^{14}-bilirubin. In all instances, a significant proportion of the radioactivity excreted in bile was identified as C^{14}-bilirubin. This proportion appeared to be greater following the administration of unconjugated C^{14}-bilirubin.

In similar experiments, bilirubin absorption was demonstrable using isolated segments, with intact blood supply, from the upper, middle, and lower third of the small intestine.

78. The diffusing capacity of the lungs in emphysema: An analog computer analysis

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During a 45 second period of rebreathing at 30 c.p.m., measurements were made in 4 patients with emphysema of volume of respiration, concentration of neon (an insoluble gas), and concentration of carbon monoxide. Residual volume was determined independently by a 7 minute helium equilibration and fractional lung volumes were measured by the nitrogen

washout method of Fowler, Cornish, and Kety (J. Clin. Invest. 31: 40, 1952), during a 7 minute period of quiet breathing and during a 45 second period of deep breathing at 30 c.p.m.

A system of a rebreathing bag and a lung of two regions was simulated with an analog computer. Known values for bag volume, bag dead space, tidal volume, and subject's dead space were used. The total volume of the two lung compartments was known from the independent measurement of residual volume. This value and the tidal volume were then distributed between the two regions in various proportions to obtain an empirical match between the mixing curve of an insoluble gas given by the analog computer and the actual curve of neon mixing in the patient. The "poorly ventilated" region received a smaller portion of the tidal volume in this empirical curve fitting than was predicted by nitrogen washout data.

Various diffusing capacities were then assigned to the two lung regions on the computer and these diffusing capacities varied until the carbon monoxide absorption curve was matched. Such a match was obtained only when the ratio of diffusing capacity to regional volume was equal in the two regions. Under these circumstances, the total diffusing capacity of the lungs calculated from the CO absorption curve was nearly equal to the sum of the diffusing capacities of the two regions.

79. Dose-response relationship for pancreozymin in the canine pancreas: Evidence for receptor sites

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In 9 acute experiments on anesthetized dogs, intravenous doses of pancreozymin (PZ, Lilly) ranging from 0.25 to 8.0 mg. per kilogram were given in randomized order at 30 minute intervals. A continuous infusion of secretin (Lilly) in saline was given at the rate of 100 u per hour. Pancreatic secretion was obtained by cannulation of the major duct. Protein content was determined by the tyrosine method and taken to represent the total output of enzymes. A portion of the uncinate process was then resected, from 13 to 33 per cent of the weight of the gland. After a recovery period the same doses of PZ were given again in the same order. With doses above 0.5 mg., plotted on a logarithmic scale, responses were linear in all 7 instances where there were multiple points above the threshold.

In 5 animals the dose-response curves after partial resection were essentially the same as before. In the remaining 4, responses were the same at lower doses, but with substantially lower output at higher doses. The differences between these dogs could not be accounted for by differences in amount of pancreas resected, pancreatic mass, or mass in relation to body weight. Since variations between dogs were so great it can be speculated that the 3 or 4 doses given to each animal embraced different parts of their respective dose-response curves.

To explain the similar curves for the entire dose range in 5 dogs, and at the lower doses in all 9, the existence of a finite number of pancreatic receptor sites is postulated. Thus it is possible to resect a portion of the gland and obtain the same response to low doses (below saturation level) because an adequate number of receptor sites would remain to accept all the available PZ. At high doses the responses in 4 dogs became flatter after resection. This can be interpreted to indicate "saturation" and insufficient receptor sites remaining to accept the PZ offered. In the 5 dogs with similar curves after resection it can be assumed that enough sites remained to utilize all the PZ, i.e., "saturation" had not occurred.

80. Tolerance to bacterial endotoxin in patients with pyelonephritis: Clinical significance

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Introduced by Paul Heller, M.D.

Many of the profound physiologic disturbances produced by the endotoxins of gram-negative bacteria are ameliorated by repeated injections of heterologous or homologous endotoxin. This phenomenon of endotoxin tolerance has not been observed during clinical or experimental infections with gram-negative bacteria. The possibility that patients with pyelonephritis are endotoxin tolerant was suggested during a review of 173 patients with gram-negative septicemia by the relatively more benign course in these patients in comparison with other patients with septicemia. Previously reported studies have indicated that the production of pyelonephritis in rabbits induced tolerance to the pyrogenic effects of bacterial endotoxin. The present investigation was undertaken to confirm these findings in humans by determining the pyrogenic response of patients with pyelonephritis and controls in intravenous *Salmonella typhosa* vaccine.

Patients with bacteriuria in excess of 10^5 gram-negative bacilli per milliliter of urine and their age-matched nonbacteriuric controls were challenged with 1×10^7 dead typhoid bacilli intravenously. Hourly rectal temperatures were determined before and for 8 hours after endotoxin administration. Leukocyte counts, plasma 17-hydroxycorticosteroid levels, and the urinary excretion of leukocytes were determined before and after endotoxin administration.

A significant diminution in the magnitude and duration of fever ($t_{22} = 5.2$; $P < .001$) was observed among patients with pyelonephritis (mean 20.9 ± 6.7) as compared with the controls (mean 41.3 ± 11.4). In addition, a positive correlation between age and the magnitude of the pyrogenic response was observed in the controls but this correlation was obscured by tolerance to endotoxin in the pyelonephritic patients.

The classic leukocyte response to endotoxin was observed in both groups of patients and no significant difference in the magnitude of response could be detected between the two groups. Timed excretion rates of leukocytes in the urine did not differ significantly between the two groups except for the greater magnitude of initial pyuria among patients with pyelonephritis. The latter findings conflict with previous reports of the diagnostic value of this test in pyelonephritis.

These results indicate that chronic pyelonephritis induces tolerance to heterologous endotoxin and help explain the relatively afebrile course of chronic pyelonephritis despite continuing renal destruction by pyrogenic bacteria.

81. Conditions affecting in vitro histamine release from allergic human blood

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Introduced by John M. Sheldon, M.D.

Several laboratories have reported that histamine is released from leukocytes into plasma in vitro upon the addition of allergens to the blood of sensitive human subjects. Maximal histamine release requires at least 30 minutes at 37° C. in the presence of calcium ions. In an effort directed toward developing a model system in which the reactants can be quantitated, several additional conditions affecting in vitro histamine release have been investigated. Histamine has been determined either by the spectrophotometric method of Lowry, Graham, and associates or by the fluorometric method of Shore and co-workers.

Since plasma diamine oxidase activity, a nonspecific plasma inhibitor, and activation of a histamine-destroying factor are known to affect measurements of histamine release by

antigen in other species, the human system was studied for these plasma effects, but they were found to be absent or negligible under the conditions used. Indeed, partial removal of plasma resulted generally in decreased histamine release. However, an important finding, confirmed by others, has been that substantial histamine release occurs from blood cells washed free of plasma with Tyrode's solution. Fourteen histamine release curves obtained with varying amounts of antigen and washed cells resemble 41 curves obtained with whole blood. Using washed cells, it has been shown that both the amount of antigen added and the concentration of antigen affect histamine release in the region of antibody excess. Kinetic studies have shown that the rate of histamine release increases with increasing cell (antibody) concentration.

Although quantitative interpretation of these data obviously is limited by the heterogeneity of the pollen extract allergens, it is of interest that similar histamine release curves have been obtained with partially purified ragweed extract fractions, e.g., Trifidin A, and with blood of a tannic acid–sensitive patient. The reproducibility of the method is such that under suitable conditions one can distinguish between quantities of allergen differing by 16 per cent, a discriminating capacity which compares favorably with that of immediate type skin test reactions. Whole pollen grains and ragweed extract dialysates have been shown to produce in vitro histamine release.

82. Quantitative histochemistry of the human nephron: The possible role of enzymes in proton and ammonia excretion

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In 1958 we reported to this Society the techniques used for the study of the quantitative distribution of alkaline phosphatase and lactic dehydrogenase activities in the individual anatomic units of the human nephron in health and disease. Enzyme activities were assayed on accurately identified and dissected glomeruli, proximal and distal convolutions, medullary rays, and medulla from frozen-dried renal biopsies. These techniques have been applied to examine the possible role of selected enzymes in ammonia production and proton excretion by the nephron.

Carbonic anhydrase (CA), glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), glutamic dehydrogenase (GDH), lactic dehydrogenase (LDH), and malic dehydrogenase (MDH) activities were assayed quantitatively in 4 healthy kidneys; in kidneys from one patient each with cystinosis, renal tubular acidosis, severe hypokalemia, and idiopathic hypercalcemia; from 2 healthy subjects made acidotic with NH_4Cl; and from 3 patients with hepatolenticular degeneration.

In healthy kidneys, GOT, GPT, and GDH activities were low in the glomeruli. Higher activities were found in the convoluted tubules, but there was no significant difference between the activities of each of these enzymes in the proximal and distal convolutions. In the abnormal kidneys, no significant deviations from normal values were found.

In health, carbonic anhydrase activity was higher in the distal than in the proximal convolution; no decrease of CA activity was found in renal tubular acidosis. This observation might possibly suggest that CA deficiency was not responsible for the relative failure of proton excretion in renal tubular acidosis.

MDH activity was the same in health in proximal and distal convolutions. MDH activity was increased significantly in the convoluted tubules of healthy subjects given NH_4Cl for 7 days, in renal tubular acidosis, and in hypokalemia, whereas the activity was decreased significantly in 2 cases of Wilson's disease with heavy aminoaciduria, in cystinosis, and in idiopathic hypercalcemia.

The exact interpretation of the differences in MDH activity is not yet clear. As this enzyme may possibly reflect over-all energy production in the tubular cell, it is pertinent to note that MDH activity was increased in conditions in which there may be intracellular acidosis.

83. Abnormal patterns of granulocyte kinetics

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Introduced by John J. Will, M.D.

Determinations of the total blood granulocyte pool (TBGP), the half time of granulocyte survival in blood ($T\frac{1}{2}$), and the granulocyte turnover rate (GTR) can be made in humans by infusing blood labeled in vitro with P^{32}-tagged diisopropylfluorophosphate and subsequently measuring at intervals the radioactivity of the isolated blood leukocytes. The mean values and standard deviation for normal subjects reported by Athens and associates in 1961 are as follows: TBGP $= 65 \pm 22 \times 10^7$ cells per kilogram; $T\frac{1}{2} = 6.6 \pm 1.2$ hours; and GTR $= 180 \pm 74 \times 10^7$ cells per kilogram per day.

Three patterns of abnormal granulocyte kinetics have been found so far in a study of patients from the Children's Hospital and the Cincinnati Veterans Administration Hospital. These three patterns are granulocytosis with increased marrow production, granulocytopenia with decreased marrow production, and granulocytopenia associated with shortened blood survival time and inadequate marrow compensation.

Marked increases in granulocyte production were found in 3 patients with polycythemia vera and 2 patients with chronic myelocytic leukemia. The TBGP's ranged from 3 to 20 times the normal value, the $T\frac{1}{2}$'s were from 7.5 to 12 hours, and the GTR's were increased 2.5 to 13 fold. One of the patients with polycythemia vera was studied again after therapy and all values had returned to normal.

Granulocytopenia resulting solely from decreased marrow production was found in 2 patients receiving vinblastine sulfate as tumor chemotherapy. The TBGP's were 20 per cent of the normal value, the $T\frac{1}{2}$'s were 9 and 12.5 hours, and the GTR's were 15 and 10 per cent of the normal rate.

Granulocytopenia associated with marked shortening of the granulocyte survival time in blood and only slight increase in marrow production was found in a patient with postnecrotic cirrhosis and congestive splenomegaly and in a patient with megaloblastic anemia. The $T\frac{1}{2}$'s were 1 and 1.5 hours, respectively, and yet the GTR's were less than twice normal. A repeat study of the patient with megaloblastic anemia after treatment was normal.

The method is a particularly useful technique for the study of granulocytopenia for which mechanisms are frequently obscure. Undoubtedly other kinetic patterns will be found with further study.

84. Comparison of individual pituitary gonadotropin preparations and reference standards from pooled urine

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Extracts of pooled human urine have been generally accepted as reference standards for the clinical bioassay of pituitary gonadotropin (HPG). Menopausal urine extracts (HMG) have been most widely used because of greater potency. Reported differences between HMG and extracts of pooled male urine have not been confirmed, but ovine gonadotropin and human

chorionic gonadotropin (HCG) do have significant biologic differences which preclude their use as reference standards for HPG.

Using the mouse uterine weight assay of kaolin extracts, the essential similarity of HMG and extracts of pooled male urine has been confirmed. However, when compared to these reference preparations, gonadotropin extracts of pooled intermenstrual urine of mature women produced a significantly different slope of the uterine dose response curve ($p = < .001$). No difference was found between pre- and postmenstrual pools ($p = 0.7$ to 0.5). Individual gonadotropins from each of 2 normal men, 3 normal women, and 4 hirsute women, prepared in the same manner as the standard pool from 10 to 20 day collections of urine, were simultaneously assayed with the reference extracts. The dose response curves of all normal individual gonadotropins differed significantly from those of the reference material. Curves of normal men and women were similar, but most differed significantly from hirsute subjects. Extracts from 2 of the 3 normal women produced significantly different responses from that of the pooled gonadotropin of the same 3 subjects. As prepared for clinical bioassay, gonadotropin extracts are not sufficiently similar to compare to a single reference standard. These individual differences are obscured when pooled urine is analyzed.

85. Studies on the regulation of iron absorption

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Introduced by William R. Barclay, M.D.

It has been suggested that there is a direct relationship between the rate of erythropoiesis and the absorption of iron from the gastrointestinal tract. Experiments were conducted in mice to explore further this relationship.

As has been reported by others, hypertransfusion of homologous red cells effectively suppresses erythropoiesis and iron absorption in mice. Hypertransfused mice, treated with erythropoietin, demonstrated an increase in iron absorption paralleling the increase in red cell production induced by erythropoietin. To determine whether the action of erythropoietin in augmenting iron absorption was a direct one on the gastrointestinal mucosa or was dependent on the acceleration of red cell production, groups of mice were splenectomized and treated with strontium^{89} to impair the capacity of the bone marrow to respond to erythropoietin. Hypertransfused mice pretreated with splenectomy and Sr^{89} administration showed no increase in iron absorption in response to erythropoietin. It was also found that mice treated by splenectomy and Sr^{89} showed little change from normal in iron absorption in spite of marked suppression of erythropoiesis.

To determine whether the reported effect of hypoxia in increasing iron absorption is dependent on acceleration of erythropoiesis, the effect of decreased oxygen tension on iron absorption in normal and splenectomized-Sr^{89}-treated mice was studied. Normal mice exhibited an increase in erythropoiesis and in iron absorption when subjected to a hypoxic stimulus. However, splenectomized-Sr^{89}-treated mice showed a substantial increase in absorption without any measurable change in the rate of erythropoiesis.

It would appear from these studies that: (1) the augmentation of iron absorption from the gastrointestinal tract induced by hypoxia can occur independently of acceleration of erythropoiesis, (2) erythropoietin is capable of augmenting iron absorption, but this property of erythropoietin is indirect, being dependent on acceleration of erythropoiesis.

86. Clinical and serologic estimates of the prevalence of rheumatoid arthritis in the population of Tecumseh, Michigan, 1959-1960

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Between February, 1959, and October, 1960, approximately 90 per cent of the 9,700 residents of Tecumseh, Michigan, participated in a community health study directed at cardiovascular and other chronic diseases and conducted by the Department of Epidemiology, School of Public Health, University of Michigan. Methods of study included: (1) an interview by a trained worker using a detailed medical questionnaire; (2) review and augmentation of the medical history by the examining physician; (3) a detailed physical examination, including evaluation of the spine and peripheral joints; (4) certain physical measurements and laboratory examinations, including latex fixation tests for rheumatoid arthritis; and (5) review of diagnostic impressions by a panel of physicians.

From the collected data it has been possible to derive age and sex specific prevalence rates for the following items: (1) history of joint pain or aching, (2) history of joint swelling, (3) history of morning stiffness, (4) previous diagnosis of arthritis or rheumatism, (5) presence of abnormal joint findings, (6) presence of Heberden's nodes, (7) presence of subcutaneous nodules, (8) results of the latex fixation test, and (9) the clinical diagnosis of rheumatoid arthritis.

The latex fixation test appeared to have relatively little value in case detection inasmuch as only one third of those with positive tests were included in the group with suspected rheumatoid arthritis. Positive results were obtained in 3.3 per cent of the females and 3.4 per cent of the males. The prevalence of positive results increased with age in both sexes.

The clinical diagnosis of rheumatoid arthritis was suspected in 7.4 per cent of females and 3.45 per cent of males, the female to male ratio of suspected cases being 2.3 to 1. The diagnosis of definite rheumatoid arthritis, as defined by the American Rheumatism Association, could be established in only 0.57 per cent of females and 0.2 per cent of males, the sex ratio for this diagnosis favoring females by 3 to 1.

87. Renal transplants in the rat

BENJAMIN F. MILLER, M.D., EDWARD E. GONZALEZ, M.D. (By Invitation), and PAUL NATHAN, PH.D. (By Invitation) *Cincinnati, Ohio*

Research with organ transplants generally has been restricted to such large species as the dog because of the difficulties encountered in surgery on small animals in which anastomoses must be carried out with arteries and veins measuring less than 1 mm. in diameter. We have performed kidney transplants in rats by direct anastomoses of the donor's aorta and vena cava to the recipient's carotid artery and jugular vein. The success of this procedure has been made possible by microsurgical techniques and instruments designed for surgery on blood vessels less than 1 mm. in diameter. Inexpensive clips for manual connection of small vessels, and also the use of watchmakers' instruments have greatly facilitated the development of this method.

Six out of 7 isologous transplants performed with inbred rats of the Lewis strain were completed successfully. Biopsies were obtained 35 days after the operation. There was no evidence of rejection in histologic sections. One rat survived 71 days after its own kidneys had been removed. This animal died of an unknown cause, but the transplant had not been rejected. Some of the hosts have died because of depressed renal function in the transplant caused by partial block of the ureterostomy. The healing of the skin over the exteriorized ureter necessitates reconstruction of the orifice approximately every 10 days.

Homologous transplants from one Sprague-Dawley rat to another show a typical transplant reaction within 1 week. However, unlike the tubules in the dog, some tubules may remain viable for 50 days after transplantation.

88. Elimination of inflammation to homografts in hosts pretreated with placental tissues

C. W. MONROE, M.D. (By Invitation), R. H. ANDRESEN, M.D., D. A. MADDEN, B.A. (By Invitation), and S. SWARTZBAUGH, PH.B. (By Invitation) *Chicago, Ill.*

It was possible to eliminate inflammation to homologous musculofascial grafts in adult nonpregnant rabbits by injection of the recipient with pooled, crushed embryos (14 to 16 days of age and 7 to 11 in number) prior to grafting. The modified homologous tissue reaction consisted of a fibrocollagenous encapsulation of the graft with varying degrees of vascular penetration by the host in the absence of customary lymphocytic infiltration. Studies during pregnancy revealed that massage of the intact uteroplacental sites of an entire litter (7 to 11) at midgestation prior to grafting of the maternal host from adult random donors frequently resulted in a modification of the host-homograft interaction similar to that observed in hosts pretreated with embryonic tissues. In view of conditioning mechanisms inherent in embryonic tissues following transplantation and the modification of the local reaction of the pregnant host to homografts following uteroplacental massage, the following experiments were designed to characterize the host-homograft interaction in adult hosts receiving placental tissues prior to grafting: rabbit placental tissues (7 to 11 per litter) were removed at midgestation under sterile precautions, pooled, crushed, and injected into the erector spinae of each recipient. Six days later, each recipient received a musculofascial graft from a random adult donor. Two weeks later, the musculofascial and placental graft sites were prepared for microscopic study. The musculofascial grafts underwent fibrous encapsulation accompanied by varying degrees of vascular penetration by the recipient. Inflammation was conspicuously absent. The placental grafts failed to proliferate as a result of a curious lack of stromal penetration by the host. These studies have shown that, although placental tissues do not proliferate following transplantation, as is true of embryonic tissues, nevertheless, placental tissues under these conditions possess conditioning mechanisms similar to those of tissues of the embryo as evidenced by their ability to alter the local reactions of the pretreated adult host to homografts.

89. Pathogenesis of hyperglycemia produced in dogs by an antiserum against insulin

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Introduced by Max Miller, M.D.

Insulin antiserum was produced in guinea pigs by the repeated administration of insulin in Freund's adjuvant. The potency of each antiserum was determined by the following methods: (1) hyperglycemic effect in rats after the injection of antiserum; (2) inhibition of convulsions in mice after insulin administration; (3) decrease of insulin effect on the oxidation of glucose-1-C^{14} to $C^{14}O_2$ and on the incorporation of C^{14} into lipids by the rat epididymal fat pad; and (4) binding capacity using I^{131}-insulin (J. Clin. Invest. 35: 170, 1956). Each method gave approximately the same results for the potency of a particular antiserum.

Thirty milliliters of antiserum capable of neutralizing about 60 units of insulin was administered intravenously to unanesthetized dogs with catheters in the hepatic artery, hepatic vein, and portal vein. Hepatic output of glucose and peripheral utilization of sugar,

therefore, could be determined. After the administration of the antiserum blood sugar gradually rose, reaching a maximum (about 250 mg. per cent) after 90 minutes. Hepatic glucose output was increased for 2 hours paralleling the rise in blood sugar. Peripheral utilization of glucose was subnormal immediately following the administration but returned to normal and even supernormal levels when the blood sugar rise was at its maximum and during its return toward normal.

In control tests, injection of normal guinea pig serum did not cause hyperglycemia or change hepatic glucose output.

90. Cyclical edema and reactive hypoglycemia

JACK H. MOSTOW, M.D. (By Invitation), GEORGE J. HAMWI, M.D., and THOMAS G. SKILLMAN, M.D. (By Invitation) *Columbus, Ohio*

All cases diagnosed as cyclical edema on the Endocrinology Service since October, 1959, a total of 27, were included. The patients presented moderate to marked cyclical fluctuation in weight unexplained by cardiac, renal, or hepatic dysfunction, nutritional deficiency, or venous insufficiency. Five hour oral glucose tolerance tests were performed on 21 patients as part of an extensive laboratory evaluation of each case.

The diagnosis of reactive hypoglycemia was made in individuals who for several days preceding glucose tolerance tests had had adequate carbohydrate intake, who displayed a fall below 60 mg. per cent at 180, 240, or 300 minutes after glucose ingestion, and in whom clear-cut signs and symptoms of hypoglycemia, such as profuse sweating, tremor, palpitations, or tachycardia were observed.

Of the 21 subjects who had oral glucose tolerance tests, reactive hypoglycemia was diagnosed in 16 (76 per cent). This group of 16 patients, with combined disorders, demonstrated a higher and earlier peak in blood glucose and a greater rate of fall in glucose levels compared to either normal subjects or those patients with cyclical edema alone. Most important as a diagnostic criterion was the observation of characteristic signs and symptoms of hypoglycemia 3 to 5 hours after ingestion of glucose.

All cases of cyclical edema were in adult women whose average age was 37. Ten patients (37 per cent) had a family history of diabetes. No abnormality of calcium-phosphorus metabolism, serum electrolytes, or urinary steroids was detected.

Aldosterone determinations were normal. An alcohol diuresis test followed by nicotine stimulation was suggestive, but inconclusive, of increased antidiuretic hormone secretion.

Psychiatric abnormality was present in all patients, with the severity of disturbance more marked in the group of 16 in whom both reactive hypoglycemia and cyclical edema were present.

It is postulated that the etiology is emotional in origin, with stimulation of the autonomic nervous system resulting in cyclical edema, reactive hypoglycemia, or both.

91. Effects of bleeding in nephrectomized dogs

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Introduced by Leon O. Jacobson, M.D.

The present study was undertaken to determine whether erythropoiesis could be stimulated by bleeding in dogs made uremic by either bi- or unilateral nephrectomy. In animals subjected to unilateral nephrectomy, the remaining ureter was implanted in the iliac vein. Animals were maintained by peritoneal dialysis.

The dogs were bled immediately after nephrectomy and again on the succeeding day, the amount of blood withdrawn being 2 per cent of body weight. Normal control animals were bled on 2 consecutive days. Blood volume was maintained by dextran infusion.

Erythropoiesis was measured by plasma iron turnover and by reticulocyte and marrow normoblast counts. Control values were determined 3 or 4 days before the operation and bleeding. Measurements were repeated 3, 5, and 7 days after the first bleeding.

In 5 normal dogs, iron turnover values averaged, respectively, 145, 190, and 263 per cent of the prebleeding value on the third, fifth, and seventh days after the first bleeding. In 5 bilaterally nephrectomized dogs the comparable results for the same days were, respectively, 29, 31, and 21 per cent, and reticulocytes and marrow normoblasts disappeared after operation despite the bleeding. Three dogs with implanted ureters showed a less striking elevation in iron turnover than did the control animals, but this last group is too small to permit definite conclusions.

These results seem to indicate that the presence of the kidney is indispensable for the maintenance of the stimulation of erythropoiesis by bleeding.

Further studies on uremic dogs with the ureter implanted in a vein will determine whether the retention of toxic products alters the erythropoietic response to anemia. This problem is presently under investigation.

92. Bilirubin clearance in the isolated perfused rat liver

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MARION F. PILLER, B.S. (By Invitation), and
LEON SCHIFF, M.D. *Cincinnati, Ohio*

The isolated perfused rat liver has been used as a tool to study bilirubin metabolism in the normal rat liver. The perfusing medium was 80 ml. of normal rat blood plus 30 ml. of saline. After allowing at least 60 minutes of perfusion following the surgical procedure, the amount of bilirubin in the perfusing blood and in the bile was determined. The bile was obtained by cannulating the bile duct during the operation, and continuous bile secretion was used as one index of viability during the procedure.

A known quantity of bilirubin was then infused over a 1 minute period and blood samples were taken at 5, 10, 15, and 60 minutes after infusion. The bilirubin concentration of the blood samples and of the bile, which was collected over the 60 minutes following infusion, was determined. The liver was weighed at the termination of the experiment and the amount of bilirubin infused per gram of liver tissue was calculated. An amount of bilirubin in the plasma at 15 minutes of greater than 0.5 mg. per cent was taken to indicate incomplete clearance by the liver.

From our data, collected from 37 perfusions, we have calculated the maximum clearance of infused bilirubin by the normal isolated rat liver to be 0.21 Gm. Studies are now in progress on the influence of cortisone and other agents on bile flow and bilirubin clearance.

93. Epidemiologic aspects of postpartum bacteriuria

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(By Invitation), LESTER PERSKY, M.D. (By Invitation), and
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Bacteriuria in the immediate postpartum interval is a common finding. However, precise data are lacking regarding incidence, possible underlying factors, and late sequelae. The present study was designed to describe, in a quantitative prospective manner, a group of women who were delivered at University Hospitals of Cleveland, the incidence of bacteriuria observed on the third postpartum day, and the relationship of high bacterial counts to certain predisposing maternal and fetal characteristics.

Consecutive patients were included for study following vaginal delivery unless a history of antepartum urinary tract infection was obtained. Data were collected concerning maternal age, race and parity, duration of hospitalization prior to delivery, infant birth weight, and the use of catheterization and antibacterial drugs in the postpartum interval. Urine samples were obtained by catheter at the beginning of the second stage of labor and on the third postpartum day. Quantitative urine cultures were performed using streak plate techniques.

High bacterial counts ($\geq$ 100,000 colonies per milliliter) were observed on the third postpartum day in 25.5 per cent of women whose initial cultures were negative ($<$ 100,000 colonies per milliliter). Bacteriuria occurred more often in those patients whose stay in the hospital prior to delivery exceeded 13 hours. The presence of large numbers of bacteria in the urine was not associated with increased maternal age, race, multiparity, or prematurity. The predominant bacterial genus isolated was Escherichia, a finding consistent with a nonhospital source of organisms.

94. Leukemia in two patients with congenital agammaglobulinemia

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Introduced by R. L. Vernier, M.D.

Association of hypogammaglobulinemia with lymphatic leukemia is well documented. This deficiency is thought to be due to replacement of normal lymphoid cells by leukemic cells lacking capacity to produce gamma globulin. The occurrence of leukemia in 2 children known to have congenital agammaglobulinemia raises questions about this association.

Case 1. After age 6 months this Caucasian boy developed recurrent pneumonia and septicemia. At the age of 2 years he developed dermatomyositis. A diagnosis of agammaglobulinemia was established when an immunochemical determination revealed gamma globulin of 16 mg. per cent. Bone marrow and lymph node biopsies showed no malignancy. The patient was started on gamma globulin replacement therapy. He continued to have progressive muscular disease and died with pulmonary infection at 4 years, 8 months of age. Autopsy revealed previously unrecognized evidence of malignant lymphoma with infiltration of liver and kidneys in a pattern consistent with lymphatic leukemia.

Case 2. A Caucasian boy, sibling of a patient with known agammaglobulinemia, was followed closely from birth. At 10 months of age his gamma globulin level was 10 mg. per cent. At 4 years of age he developed fever, lymphadenopathy, and a large anterior mediastinal mass. The white blood count was 240,000 and the bone marrow showed acute lymphocytic leukemia. The patient died 4 months later. Autopsy revealed extensive leukemia infiltrates throughout the body.

These cases raise interesting possibilities. Perhaps leukemia occurring in a patient known to have agammaglobulinemia is another expression of the alteration of reticular tissue that represents the fundamental lesion in agammaglobulinemia. Perhaps defense against leukemia depends on integrity of the immunologic mechanisms, either by preventing invasion of the virus responsible for production of leukemia or by the recognition and destruction of mutant malignant cells. The occurrence of leukemia in 2 congenital agammaglobulinemic children is a provocative association that must be considered in interpreting the nature of either disorder.

95. Skin testing with penicillin derivatives of inapparent immunogenicity

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In preparing to evaluate protein conjugates containing penicillenyl or penicilloyl groups as elicitors of the wheal and erythema skin response in human subjects sensitive to penicillin, we were anxious to avoid the possibility of stimulating the production of antibody specific for these determinants. Since low molecular weight substances are generally not immunogenic, a series of compounds of graded molecular size were prepared and

compared with respect to ability to induce antibody formation and to elicit immediate skin responses. The substances examined contained as determinants either the dinitrophenyl, penicilloyl, or penicillenate group.

Immunogenicity was evaluated by injecting groups of 5 guinea pigs with 1 mg. of test substance in Freund's adjuvant. Sera obtained at 2 weeks were individually tested for antibody by precipitin assay and passive cutaneous anaphylaxis in guinea pigs. The capacity to elicit immediate skin responses was studied in actively and passively sensitized human subjects and by passive cutaneous anaphylaxis in the guinea pig. The results with all 3 determinants were similar.

Bivalent compounds (i.e., with 2 determinants per molecule) of molecular weight less than 1,500 failed to evoke formation of detectable amounts of serum antibody. Polylysine derivatives of molecular weight up to 100,000 containing 80 to 90 determinants per molecule likewise had no detectable immunogenicity. Protein conjugates produced hapten-specific antibodies in high titer, as expected. Hapten-substituted polylysines and the correspondingly substituted protein conjugates were about equally effective in evoking immediate skin responses. Bivalent substances were effective only in certain instances. Preliminary skin testing of human subjects (collaboration with Dr. Shapiro, United States Public Health Service) indicates that the penicilloyl group is an important antigenic determinant in human penicillin sensitivity; sensitivity to the penicillenate group is less common but does occur, with or without coincident penicilloyl sensitivity.

96. The distribution and excretion of insulin in diabetics and nondiabetics with the nephrotic syndrome

JOHN R. PETERSEN, M.D., and NORMAN H. ENGBRING, M.D.
Milwaukee, Wis.

Introduced by William W. Engstrom, M.D.

Decreasing insulin requirements and increased sensitivity to insulin often characterize the diabetic individual who develops the nephrotic syndrome. Decreased food intake, secondary pituitary failure, diminished inactivation of insulin by diseased kidneys, etc., have been proposed as explanations for this phenomenon. Insulin-treated diabetic patients develop insulin-binding antibodies. Since these antibodies may be related to increased insulin requirements and since they may be excreted in the urine of nephrotic patients, it seemed appropriate to study the distribution of labeled insulin in normal persons, diabetic subjects with and without the nephrotic syndrome, and nondiabetic nephrotic patients.

Blood levels of trichloracetic acid precipitable radioactivity 30 minutes after intravenous injection of I^{131}-insulin were highest in the diabetic patients and lowest in the nondiabetic nephrotic subjects. These findings indicate intravascular binding of insulin in the diabetics as well as expanded extravascular volume ("insulin-space") in the nondiabetic nephrotic patients. During the interval from 30 to 120 minutes the blood levels dropped rapidly in normal subjects and at comparable slow rates in both diabetic and nondiabetic nephrotics. These findings were probably related to a slow return of insulin into the blood from the expanded extravascular volume in the nondiabetic nephrotics.

Using a method of selective precipitation of bound insulin, proteins having insulin-binding capacity were demonstrated in the blood and urine of diabetic nephrotic subjects. One nondiabetic nephrotic patient excreted protein in the urine with insulin-binding capacity (? nonspecific); however, no insulin binding was demonstrated in the blood.

Excretion of trichloracetic acid precipitable radioactivity in the urine (either protein-bound or free insulin) was directly related to the degree of proteinuria in both diabetic and nondiabetic subjects, and persisted for as long as 10 hours after the dose was given. Nonnephrotic patients excreted no significant precipitable radioactivity. The findings indicate that insulin-binding protein is lost in the urine of insulin-treated diabetic nephrotic patients; this loss may partially explain increased insulin effectiveness when the nephrotic syndrome develops.

97. The effects of potential myelotoxic drugs upon nucleic acid synthesis of bone marrow cells in vitro

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With the technical assistance of JOYCE KALDAHL

The effects of chlorpromazine (CPZ), chloramphenicol (CAP), and mesantoin (MSN) on nucleic acid synthesis by marrow cells were observed in short term culture preparations. Tritiated thymidine and uridine were used to identify synthesis of desoxyribose nucleic acid and ribose nucleic acid, respectively. The number and types of bone marrow cells which incorporated these compounds after 4 hours of incubation were identified by radioautography. The results were related to the type and concentration of the drugs studied.

Observations were made on marrow from 25 subjects who had never had aplastic anemia or agranulocytosis, 8 subjects who had recovered from agranulocytosis (CPZ), and 2 subjects who had recovered from aplastic anemia (MSN).

Chlorpromazine significantly inhibited the incorporation of H^3-thymidine and uridine into granulocytes in all subjects who had had agranulocytosis (CPZ) and in 18 of 25 "normal" subjects. Incorporation by normoblasts was not affected. Chloramphenicol depressed the incorporation of H^3-uridine and, to a lesser extent, thymidine into both granulocytes and normoblasts in 70 per cent of all marrow preparations. Mesantoin depressed uridine incorporation into all cells of the 2 subjects who had recovered from aplastic anemia due to this drug to a greater extent than that observed in "normal" control subjects.

Nonmyelotoxic drugs, such as aspirin, Phenergan, and penicillin in comparable molecular concentration did not affect nucleic acid synthesis. The concentrations of all test drugs used in these in vitro experiments did not influence viability or function of marrow cells as evidenced by motility, phagocytosis, redox potential, protein synthesis, and cell respiration.

The results of these in vitro experiments indicate that chlorpromazine, chloramphenicol, and mesantoin inhibit nucleic acid synthesis by bone marrow cells and, in the case of chlorpromazine, that only those cells (granulocytes) known to be affected in clinical toxicity are so inhibited. No qualitative differences were observed between subjects previously known to be "sensitive" to the test agents and "normal" control subjects.

98. Occurrence of hemophilia and von Willebrand's disease in the same family

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Milwaukee, Wis.

The two most common hereditary bleeding diseases are hemophilia and von Willebrand's disease (also known as pseudohemophilia A). The incidence of the two diseases is sufficiently high to make it probable that both might occur in the same family. Such an expectation has recently been realized. The mother of a son with severe hemophilia has one sister who had two boys who died of bleeding. Their disease was diagnosed as hemophilia. A third sister has the typical findings of von Willebrand's disease. She bruises easily, had frequent epistaxes during childhood, has menorrhagia, and has bled severely post partum. The bleeding time is prolonged, the tourniquet test positive, but the other hemostatic tests, including the clotting time, prothrombin time, prothrombin consumption time, thromboplastin generation test, clot retraction, and platelet count, are normal. One of her sons and a daughter also have a history of bleeding, while one son is normal. The type of bleeding, the hereditary pattern, and the laboratory tests correspond to von Willebrand's disease.

The father of these sisters is normal but the mother has the characteristic bleeding of von Willebrand's disease: easy bruising, nose bleeds, menorrhagia, and postoperative bleeding. In addition to having this disease, she is also a carrier of the hemophilic gene.

The occurrence of von Willebrand's disease and hemophilia in one family is of both theoretical and practical importance. Such a dual defect may perhaps explain several

of the puzzling pedigrees reported before accurate diagnostic methods were available. It is likely that some of the cases diagnosed as hemophilia in females with hemophilic descendants actually were von Willebrand's disease. The possibility exists that an individual belonging to a family such as is reported may inherit both diseases and that if this should occur, a severe bleeding state would result.

99. Effect of prednisone on the hypercalciuria of Wilson's disease (hepatolenticular degeneration)

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Previous studies suggest that hypercalciuria in some patients with Wilson's disease is secondary to increased absorption of calcium from the intestinal tract. Since corticosteroids may correct the excessive absorption of calcium and the hypercalciuria associated with sarcoidosis, the effect of prednisone was studied in 2 patients who had Wilson's disease and hypercalciuria. Both patients had been taking 250 mg. of DL-penicillamine three times a day for more than a year. This regimen was continued during the study. Calcium balance was studied for several 6 day periods before, during, and after the administration of prednisone, which was given orally in divided doses totaling 45 mg. per day for two periods, followed by one period during which the dose was reduced from 20 to 10 mg. per day. One patient, who ingested 935 mg. of calcium in the diet daily, excreted an average of 468 and 435 mg. of urinary calcium and 454 and 458 mg. of fecal calcium per day during two control periods. While the patient was receiving prednisone, the excretion of urinary calcium averaged 487, 535, and 523 mg. and that of fecal calcium, 361 and 275 mg. per day. During two subsequent control periods, the urinary calcium averaged 324 and 303 mg. and the fecal calcium, 484 and 571 mg. per day. The second patient ingested 1,243 mg. of dietary calcium daily and excreted an average of 322 and 315 mg. of urinary calcium and 868 and 834 mg. of fecal calcium per day for two control periods. With prednisone, the daily urinary calcium averaged 405, 473, and 489 mg. and fecal calcium averaged 777, 682, and 587 mg. During two subsequent control periods, the urinary calcium averaged 381 and 350 mg. and fecal calcium, 831 and 818 mg. per day. Both patients remained in a slightly positive calcium balance during the entire study. Prednisone caused some increase in the urinary excretion of calcium as well as some increase in the absorption of calcium from the intestinal tract—an effect opposite to that seen in sarcoidosis.

100. The specific adaptation rationale of injection treatment of inhalant allergy

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Since the effectiveness of injection therapy of pollinosis and related conditions has been neither adequately measured nor explained immunologically, another interpretation may be helpful.

Most persons exposed to airborne pollens, molds, dusts, animal danders, insect debris, and other biologic particles adapt to them (Stage II) without apparent ill effects. But in the presence of increasing susceptibility, the stages of adaptation to specific incitants are accelerated and clinical effects accentuated. Failure of adaptation to sustained specific exposures (Stage III) is associated with the emergence of *chronic* symptoms.

With low degrees of individual susceptibility, reactive symptoms may occur only following massive exposures, such as at the peak of the ragweed season. But with high degrees of susceptibility, reactions may occur from infinitely small amounts, as during the first

days of a given pollen season. Specific adaptations are usually multiple. Cross-reactions are common. For instance, the person susceptible to ragweed pollen and cantaloupe may manifest respiratory or gastrointestinal symptoms only when concurrently exposed.

Manifestations associated with a relative exhaustion of adaptation may be treated effectively by graded doses of specific extracts. Parenterally administered intermittent regular injections apparently counteract allergic manifestations by inducing a more potent endocrine-electrolyte response than occurs spontaneously from the inhalation of more sustained amounts.

Such a relatively symptom-free specifically adapted response (Stage II) is best induced by starting with small doses of biologic extracts within the approximate range of the end point of reaction as determined by serial dilution skin testing. It is maintained by relatively constant-sized to gradually increasing regularly spaced injections.

Although good clinical results may be obtained by regular injections at various dosage levels and intervals, chronic symptoms are relieved most effectively by minimal specific amounts at maximal intervals. This program not only avoids cumulative effects but also reduces the incidence of *acute* localized or systemic reactions. These consist of nonadapted Stage I responses requiring sharp reductions in subsequent dosage, and nonadapted Stage III responses which contraindicate further specific injection therapy. These immediate reactions must be differentiated from each other as well as from nonadapted responses to chemical preservatives and related exposures.

101. Correlation of intracellular potassium concentration with extracellular electrolyte metabolism during the course of decompensated cirrhosis of the liver

ALEXANDER P. REMENCHIK, M.D. (By Invitation), PETER J. TALSO, M.D., and EDWARD O. WILLOUGHBY, M.D. (By Invitation) *Hines, Ill.*

This study was designed to test the hypothesis that an intracellular deficit of potassium occurs in some patients suffering from decompensated cirrhosis of the liver and that this deficit could be predicted from serum levels of potassium, sodium, pH, chloride, or bicarbonate. Total exchangeable potassium, tritium space, and radiosulfate space were measured serially in 14 individuals with decompensated cirrhosis. Intracellular potassium concentration was calculated from these data. While serum levels of potassium, sodium, pH, and bicarbonate could be predicted from the intracellular potassium concentration, the converse was not true. The clinical course of the patients did parallel the intracellular potassium concentration. The less severely ill patients had levels in the normal range (100 to 125 mEq.) while 3 of 4 patients who had values below 85 mEq. per liter died. The clinical implications of these observations will be discussed.

102. Immunoglobulin determination in normal and disease states

STEPHAN E. RITZMANN, M.D. (By Invitation), and WILLIAM C. LEVIN, M.D. *Galveston, Texas*

Application of modern immunoglobulin concepts requires simple and reliable assay techniques. Chemical and paper electrophoretic methods do not measure immunologic activity of the immunoglobulins; immunoelectrophoretic and immunokrit methods are cumbersome and/or poorly reproducible.

An immunophoretic method using Ouchterlony plates permitting diffusion between antiserum and serially diluted serum was developed. End points, expressed as titers, revealed

by the faintest reaction lines provided quantitation of gamma, beta-2-A, and beta-2-M globulins. Factors including concentration and volume of agar gel, test serum and antiserum, temperature, humidity, and distance between wells were kept constant.

Results. The end point is best observed 42 to 48 hours following application of sera, remaining stable thereafter. One hundred fifteen sera from healthy persons and patients with a variety of diseases were examined. Gamma globulin titers ranged between 1:512 and 1:2,048 in 101 patients; 14 samples revealed moderate to marked deviation: 6 samples (multiple myeloma, lymphoma, malabsorption syndrome) showed increases and 8 samples (chronic lymphocytic leukemia, multiple myeloma, and agammaglobulinemia) decreases of the titers. Increased susceptibility to infections was associated with the latter group. Beta-2-A globulin assay revealed essentially the same pattern. Increased titers were associated with acute myelomonocytic leukemia, lymphoma, and multiple myeloma; decreased titers with agammaglobulinemia, multiple myeloma, chronic lymphocytic leukemia, macroglobulinemia Waldenström, and nephrotic sydrome. In beta-2-M globulin assay the normal range is less well defined, the titers ranging between 1:128 and 1:1,028. Sera from 3 patients with macroglobulinemia Waldenström revealed titers in excess of 1:32,000; decreased titers are associated with chronic lymphocytic leukemia. Commercially available gamma globulin concentrates (Squibb) showed gamma and beta-2-A globulin titers higher than 1:32,000, but beta-2-M titers of 1:256. Cross-reactions did not interfere.

This method offers a reproducible and practical assay method for immunoglobulins in the detection of functional hypogammaglobulinemias and antibody deficiency states in multiple myeloma, macroglobulinemia Waldenström, leukemia, and related disorders. Indications for gamma globulin therapy can be better defined. A clearer distinction between certain cases of multiple myeloma (gamma and beta-2-A types) and macroglobulinemia (e.g., atypical macroglobulinemia) is afforded since multiple myeloma sera tend to react strongly either with gamma or beta-2-A globulins whereas macroglobulinemia sera react strongly with beta-2-M globulins.

103. Pressor response to contraction of ischemic muscle

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When blood flow to the arm is occluded by tourniquet and the forearm muscles are then exercised, localized pain and fatigue appear. Such exercise also produces a progressive rise in blood pressure, as demonstrated in more than 300 tests.

The pressor response in normal subjects averaged about 30/15 mm. Hg and this returned to control values within 2 minutes after restoration of circulation. Patients with diastolic hypertension or a history of fluctuating pressure had rises of as much as 80/50 mm. Hg; in these, the pressor response persisted for 5 minutes or more. Patients with diagnoses of arteriosclerosis showed marked rises in systolic pressure with relatively little rise in diastolic values. A small initial pressure rise occurs prior to the onset of pain, apparently in response to increased central nervous system activity. The pain sensation and the delayed pressor effect appear to be associated with the local accumulation of Lewis' "p" factor. This material stimulates nociceptors and probably elicits a vasoconstriction and increased cardiac rate and output. The results indicate that the ischemic contraction test has value in the examination of the responsiveness of blood pressure–regulating mechanisms.

104. Familial patent ductus arteriosus

FRANCIS F. ROSENBAUM, M.D., ROBERT F. THIMMIG, M.D. (By Invitation), and PAUL F. HAUSMANN, M.D. (By Invitation)
Milwaukee, Wis.

A family has been observed in which each of the 4 children had a patent ductus arteriosus which was treated successfully surgically. A fifth child, still less than 1 year of age, has

clinical features strongly suggesting the same anomaly. A survey of the literature and several personal communications have yielded a total of 38 instances in which 2 or more siblings had a patent ductus arteriosus. The family reported here is the largest group recorded with an established diagnosis.

The children were operated upon at ages 1, 2½, 4, and 6 years. Two of the children had evidence of a so-called malignant ductus arteriosus. Two of the children are heterozygous twins. The examination of one of them at age 2½ years, when his twin was operated upon, disclosed only a faint, apical, systolic murmur. However, 1½ years later, when he was admitted to the hospital with bronchopneumonia, the clinical features of a ductus arteriosus were present.

The parents of the children are normal. The paternal grandparents were first cousins and the paternal grandfather is reputed to have a cardiac defect. A paternal uncle of the children had a large ductus arteriosus with pulmonary hypertension; he, in turn, has a son who quite certainly has a patent ductus arteriosus. A second paternal uncle is also believed to have congenital heart disease and he, too, has a son with a congenital cardiac anomaly. A paternal aunt has had 14 children of whom one died with congenital heart disease and another is said to have a congenital cardiac anomaly.

The observations reported lend support to the belief that in some instances of patent ductus arteriosus there is a significant genetic element in the etiology of the disorder. In this particular instance, it is probably an irregular dominant. Further, the occurrence of severe symptoms at an early age in 2 of the 5 siblings tends to confirm the opinion previously recorded that familial patent ductus arteriosus is apt to be a more troublesome disorder and associated with a ductus of larger caliber with a larger shunt than is true of the more usual patent ductus Botallo.

105. Antacid effects of antacids, diets, and anticholinergic drugs recorded by in situ glass electrode in patients with active duodenal ulcer

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Introduced by Hugh R. Butt, M.D.

The pH of the gastric contents in 10 patients was examined on 15 occasions, and that of the duodenal contents in 9 patients on 13 occasions over 3 to 9 hours, including the postcibal period. Postcibal recording is particularly feasible with the glass electrode and is impractical and seldom reported with aspiration techniques. Elevations of pH in either duodenum or stomach were less than those commonly reported by aspiration techniques or from other studies using shielded glass electrodes. After administration of aluminum and magnesium hydroxide gels, the gastric pH was maintained at more than 3.5 for only 0.3 minute. Optimal anticholinergic premedication increased the duration of this neutralization fourteenfold. Ingestion of milk or cream resulted in similar minor increases in the gastric pH, but response to food was variable and unimpressive. Rapid gastric emptying is considered a possible explanation for the brief and minor neutralizing effects. Duodenal bulb pH was found on occasion to be as low as 1.5, especially after meals. The postcibal pH was occasionally about 1 pH unit higher after ingestion of milk or aluminum hydroxide than before, but the effects were often difficult to analyze because of the rapid and wide variations in pH. These variations in duodenal bulb pH were stabilized by antacids and anticholinergic drugs but increased after food and thus seemed to be physiologic, and not due to an artifact, as some have considered them. Ulcer pain occurred when the duodenal pH was 6.05; it disappeared at pH 6.38 after ingestion of antacids. Fluoroscopy with contrast medium and frequent roentgenograms were essential for accurate maintenance of electrodes in the duodenal bulb.

Despite the clinical impression that neutralization of acid is helpful in management of ulcers the changes as measured by this technique are small.

106. Systemic and coronary hemodynamic effects of a large arteriovenous fistula

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An acute arteriovenous fistula was created by placing a plastic tube between the aorta and the inferior vena cava in a series of 9 anesthetized mongrel dogs. Determinations of cardiac output (Fick principle) and coronary blood flow (N_2O method) were made with the fistula open and closed. Studies were alternated so that in some the fistula was open first and in others closed first, hence the effects of the anesthetic and the surgical procedure could be minimized. Opening of the fistula was accompanied by a considerable decrease in mean systemic arterial blood pressure, an increase in heart rate and mean pulmonary arterial blood pressure, narrowing of the systemic arteriovenous differences for oxygen and carbon dioxide, increase in cardiac output, and decrease in systemic and pulmonary resistances to blood flow, as well as increase in right and left ventricular work.

In the coronary circulation, on opening of the fistula, there was widening of the arteriovenous difference for oxygen and carbon dioxide, accompanied by a considerable increase in coronary blood flow and myocardial oxygen consumption. Coronary vascular resistance was considerably reduced.

These results clarify the general hemodynamics of arteriovenous fistula and indicate that in the essentially intact animal the coronary circulation can adapt itself to an increased work load even in the presence of a lower perfusion pressure, to supply more blood and oxygen to the heart. They also indicate that even though the coronary sinus oxygen content is low, the myocardium can increase oxygen extraction to help meet its demands.

107. The effects of age, sex, and season on the luteinizing hormone content of the human pituitary

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Introduced by Louis Limarzi, M.D.

Although the presence of luteinizing hormone (LH) in the human pituitary gland has long been recognized, very few quantitative data are available concerning LH concentration and the factors that affect it.

Pituitaries were obtained from 15 females and 19 males who died accidentally. Homogenates of individual glands were assayed for LH, using the ovarian ascorbic acid depletion method of Parlow (Human Pituitary Gonadotrophins, Springfield, 1961, Charles C Thomas, Publisher). All determinations were performed by the simultaneous assay of two doses of unknown and two doses of a standard LH preparation (NIHLHS No. 1). The dose-response relationships of the unknowns and standard were parallel. Results are presented in Table I.

Table I

Sex	*Age (years)*	*No.*	*Mg. NIHLHS No. 1/gram wet pituitary ± standard error*
Males	13-16	2	0.45 ± 0.45
	19-40	10	1.48 ± 0.18
	50-88	7	2.15 ± 0.40
Females	.09-5	4	0.37 ± 0.11
	24-36	5	2.47 ± 0.79
	48	2	3.16 ± 4.23
	58-86	4	6.32 ± 1.98

Other assays on pooled lots of acetone-dried human pituitaries over the past 2 years have indicated a seasonal variation in LH concentration. Maximum concentrations were found in late fall and early winter and minimum values during summer months. The peak pituitary LH activity in late fall-early winter, fits closely with the late summer peak in the human birth rate and suggests that there may be a human breeding season, albeit vestigial, similar to that seen in lower animals. Since pooled lots of pituitaries were used, fortuitous admixture of LH-rich postmenopausal pituitaries with LH-poor male pituitaries remains a possibility that is still under investigation.

108. The gastric smear for acid-fast bacilli in the presumptive diagnosis of tuberculosis

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(By Invitation) *Columbus, Ohio*

The gastric smear for acid-fast bacilli generally has been regarded as an unreliable and unacceptable diagnostic aid in suspected tuberculosis. The "high incidence" of saprophytic acid-fast bacilli in gastric contents has served as the basis for this contention. In a general hospital where a more rapid patient turnover is desirable, there is a need for methods to expedite the diagnosis of tuberculosis. Frequently, satisfactory sputum specimens are not readily available if the patient is comatose, disoriented, or uncooperative because of various reasons including old age, debility, or alcoholism.

For the past ten years gastric smears have been utilized in this institution as part of the diagnostic workup in cases in which tuberculosis was considered in differential diagnosis.

Acid-fast bacilli were seen in 134 of 475 (28.2 per cent) gastric smears obtained from 144 patients with subsequently proved tuberculosis. Only 1 of the 475 smears (0.02 per cent) showed a saprophyte on culture. In 214 patients with nontuberculous pulmonary disease only 4 of 573 (0.7 per cent) gastric washings showed acid-fast bacilli on smear which were saprophytes on culture. Thus, in a total of 1,013 smears from a total of 348 patients with tuberculous and nontuberculous pulmonary disease, only 5 (0.5 per cent) false positive gastric smears were obtained. In 27 of 144 (18.8 per cent) patients with proved tuberculosis, the gastric smear was positive, while sputum smears showed no acid-fast bacilli. These data support the use of gastric smears as a valuable ancillary tool in the presumptive diagnosis of tuberculosis.

109. Intravenous injections of methimazole (Tapazole) in the treatment of thyrotoxicosis

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Introduced by E. P. McCullagh, M.D.

Although the usual forms of therapy for thyrotoxicosis are adequate in most instances, the use of intravenous injections of an antithyroid drug may occasionally be useful. Lugol's solution and sodium iodide may be used intravenously, but usually are not adequate to bring the patient to the euthyroid state.

If a patient is receiving one of the antithyroid drugs for the control of thyrotoxicosis and has an intercurrent illness or undergoes a surgical operation, he may be unable to receive oral therapy for a number of days. When a patient has severe thyrotoxicosis with nausea and vomiting, intravenous injections of an antithyroid drug are also useful in initiating treatment.

Five patients were treated with intravenous injections of methimazole. The usual dose was 40 mg. with 0.5 Gm. of sodium iodide every 8 hours in 500 ml. 5 per cent dextrose. Two patients each with thyrotoxicosis had a duodenal ulcer with vomiting. The vomiting was

so persistent that an intravenous preparation was necessary. Two patients on courses of antithyroid drug therapy required major operations: one for carcinoma of the lung, and the other for carcinoma of the colon. The fifth patient had thyrotoxic heart disease with severe weakness, nausea, and vomiting. Radioiodine was not used because of persistent vomiting, and oral administration of drugs was not feasible for the first 10 days of treatment.

In each instance the thyrotoxicosis was as rapidly controlled as one would anticipate with adequate oral therapy. The patients who were euthyroid before the major operation remained euthyroid throughout the period of treatment.

110. Long-term management of Cushing's syndrome with "medical adrenalectomy" induced by triparanol (MER/29)

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Triparanol (MER/29) rapidly depresses excretion of 17-hydroxycorticosteroids (17-OHCS), 17-ketosteroids, and aldosterone in hyperfunctioning adrenal states (J. Clin. Invest. 40: 1063, 1961). In 6 patients with Cushing's syndrome, adrenocortical suppression has been maintained up to 5 months with triparanol, and clinical manifestations have markedly resolved.

Four patients with bilateral hyperplasia received 2,000 mg. daily. In 3 individuals, baseline urinary 17-OHCS between 10.1 and 21.5 mg. per day became normal on Day 1, and on Day 4 in the other. Reduced dosage maintained normal 17-OHCS excretion for 5 months in 2 patients, in whom Cushingoid manifestations disappeared in one and greatly subsided in the other. Two patients with metastatic adrenocortical carcinoma had baseline 17-OHCS of 25 and 26 mg. per day. One patient received 2,000 mg. triparanol daily for 5 months; 17-OHCS plummeted to 11.8 mg. on Day 3, but did not become completely normal until Day 21. The other patient received 4,000 mg. triparanol daily for a month; 17-OHCS fell to 13.8 mg. on Day 5, to 7.2 mg. on Day 10, and then remained normal. Concentrations of ACTH in plasmas from these patients will be reported. Triparanol suppression was reversible, since 17-OHCS rebounded to control levels within a week after treatment was stopped. In addition, spontaneous infection and febrile stress induced with Lipexal evoked suboptimal adrenocortical responses despite triparanol therapy.

Routine determinations of "serum cholesterol" showed initial decrease, then restoration of pretreatment values. Gas chromatographic fractionation of the desmosterol-cholesterol complex in serum revealed 26 to 43 per cent of total sterols to be desmosterol.

Two patients developed ichthyosis. Skin biopsies from both these and nonichthyotic patients are being analyzed for the demosterol:cholesterol ratio.

These data suggest that triparanol rapidly induces a "medical adrenalectomy" which is maintainable and reversible. In tumorous Cushing's syndrome, its preoperative use may strengthen the patient for operation. In bilateral hyperplasia, pituitary irradiation combined with triparanol suppression may constitute more rational and sequelae-free therapy than total adrenalectomy, the present treatment of choice.

111. Physiologic correlations in pulmonary fibrosis

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Introduced by Harold M. Schoolman

As part of a continuing study of pulmonary fibrosis 20 normal subjects and 30 patients with pulmonary fibrosis have been studied. Function tests included assessment of the total lung capacity and subdivisions, inspired air distribution, diffusion, and mechanics. Generally, in pulmonary fibroses, lung volumes, compliance, and diffusing capacity are lowered.

In the normal group, the resting diffusing capacity was not related to lung volumes, height, or age. However, in those with fibrosis the diffusing capacity was related to lung volumes ($p < .01$; $r = .43$ to $.54$), although it correlated poorly with compliance ($p > .05$, $< .10$; $r = .25$). In both normal and fibrotic groups lung volumes and pulmonary compliance correlated significantly ($p < .01$ to $.001$; normal patients, $r = .54$ to $.63$; fibrotic patients, $r = .72$ to $.78$). However, the relationships differed in the two groups to the extent that compliances in the fibrotic patients averaged only half what would be normally predicted from their lung volumes. Hence compliance in disease states cannot be accurately predicted from the normal lung volume to compliance relationships.

Mead and Otis and their associates showed that the evenness of gas distribution in the normal lung occurs because all terminal lung units have similar mechanical time constants (T. C. = compliance times resistance). Their concepts led us to evaluate (1) the mechanical time constant of the whole lung, and (2) the fall in lung compliance during rapid breathing (100 B. P. M.). Mead has showed that compliance falls at rapid breathing rates in patients with unevenness in mechanical function in the lungs.

The lung mechanical time constant (pulmonary compliance times pulmonary resistance) gave little useful information concerning lung function. It correlated poorly with evidence of maldistribution of inspired air ($r = 0.02$; $p > .1$). However, the percentage fall in compliance during rapid breathing did correlate with evidence of abnormal distribution of inspired air ($r = 0.52$; $p < .001$). These results support the concepts of Otis and Mead referred to above.

112. Treatment of thyrotropic exophthalmos with dextro-thyroxine

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A study has been in progress for two years, in which 20 patients have been treated with dextro-thyroxine for thyrotropic exophthalmos.

Although there is evidence that dextro-thyroxine has only approximately one half the TSH-inhibiting effects of its levo-rotating isomer, it may be given in much larger doses to patients without significantly altering their metabolic rates.

Treatment was begun only after thorough evaluation of thyroid function by the conventional methods. Each patient was followed clinically and with serial ophthalmometric measurements.

The patients fall into three groups. One group consists of those who have had previous therapy which included orbital decompression, pituitary irradiation, and corticosteroids, as well as thyroid extract. Another group consists of patients who were hyperthyroid at the time of their first examination. These were treated by conventional methods until they were euthyroid, and then d-thyroxine was used. The third group was euthyroid upon first examination and these were treated with d-thyroxine alone.

The results will be discussed in relation to the etiology and pathogenesis of the eye lesion.

113. Effect of intra-articular serotonin and competitive amines on connective tissue proliferation of rabbit joints

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There is clinical and experimental evidence to implicate serotonin and other amines concerned with its metabolism in proliferation of fibrous tissue.

In an attempt to induce proliferative changes in the knee joints of rabbits, serotonin and competitive amines dissolved in 0.5 ml. of normal saline were injected daily into the right knee joint of young adult rabbits for varying periods of time while 0.5 ml. of normal saline was injected into the left knee joint as a control. The transverse diameters of these joints were measured before and after the treatment period for any changes in size. The animals were sacrificed and joint tissues obtained for pathologic study. The period of daily injections varied from 10 to 56 days.

The amines studied for proliferative effect included serotonin, iproniazid, and isoniazid. Iproniazid is an inhibitor of the enzyme monoamine oxidase involved in the degradation of serotonin; isoniazid is closely related to iproniazid chemically but has little if any effect on monoamine oxidase.

Insignificant differences in the size of the treated joints over the control joints were observed with serotonin (total dosage 50 to 480 mg. in 13 animals) or isoniazid (total dosage 750 mg. in 14 animals). Contrastingly, marked increases in the size of the treated joints were observed with iproniazid. The increase in size was approximately proportional to the total dose given; with 750 mg. total dose an average increase of 7.3 mm. was observed in 11 animals.

With the dosage of serotonin used, definite hyperplasia of both the mesothelial and the connective tissue of the synovalis showed proliferative changes of a chronic granulomatous type with new capillary formation and increased fibroblastic proliferation. Similar but more pronounced changes were observed with iproniazid. The synovial membrane and joint capsule measured 10 to 12 times the thickness of the control joint, and giant cells, fibrinoid degeneration, and pannus formation were demonstrated. Changes with isoniazid were similar but of lesser degree.

Whether the marked changes observed with iproniazid are related to an effect on serotonin metabolism or whether these are related to an intrinsic fibrogenic property of its chemical structure is unresolved at present.

114. Hypercoagulability and thrombophlebitis

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Recent studies have shown an accelerated generation of plasma thromboplastin in 53 per cent of 30 patients with idiopathic recurrent thrombophlebitis. A similar acceleration was also observed in 80 per cent of 15 patients with cancer of the lung and 100 per cent of 17 patients who had undergone operation for nonmalignant disease, although none of these patients had clinically evident thrombophlebitis. Investigation of the cause of the accelerated thromboplastin generation revealed two distinct mechanisms.

In the patients with idiopathic recurrent thrombophlebitis, accelerated thromboplastin generation was unrelated to the presence of active thrombophlebitis and unaffected by coumarin anticoagulant therapy. It was associated with a nondialyzable fraction of oxalated plasma, which accompanied the beta globulins electrophoretically and precipitated from plasma at an ammonium sulfate saturation of 40 per cent. Failure to demonstrate this activity in serum and its persistence after barium sulfate adsorption distinguishes the accelerator from factors VII (stable), IX (PTC), X (Stuart), and from prothrombin. The accelerator was more stable at 56° C. than factors V (labile) or VIII (AHG). We have, therefore, ascribed the phenomenon of accelerated thromboplastin generation in patients with idiopathic recurrent thrombophlebitis to an excessive amount of a newly recognized clotting factor—thromboplastin generation accelerator (TGA)—which is present in lesser amounts in normal plasma.

Unlike the patients with idiopathic recurrent thrombophlebitis, postoperative patients and patients with pulmonary malignancy who had accelerated thromboplastin generation did not manifest increased TGA activity. Like TGA, the accelerator in the latter patients was not removed from plasma by adsorption with barium sulfate, it was absent from serum, and it was nondialyzable. Unlike TGA, it accompanied gamma globulins electrophoretically, precipitated from plasma at an ammonium sulfate saturation of 25 per cent, resisted aluminum hydroxide adsorption, and was more heat labile than TGA. The accelerator is similar, perhaps identical, to factor VIII (AHG).

Excessive amounts of factor VIII (AHG) or TGA can accelerate the generation of plasma thromboplastin. While superficially similar, these accelerators differ in their physicochemical properties. Clinically, it seems likely that an excessive concentration of either factor may be related to thrombosing tendencies.

115. An experimental autoimmune model for the regular production of a fatal glomerulonephritis in sheep

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Introduced by Ernst J. Plotz, M.D.

Although the etiology of human glomerulonephritis is unknown, certain clinical observations and experimental studies indicate it may well have an autoimmune basis. The crucial step postulated in the autoimmune theory is that the diseased person himself should produce an immunologic response mediated by autoantibodies or antibody-bearing (sensitized) cells or both, specifically directed at an antigenic component of his own kidney (glomeruli) and capable of initiating injury to the antigen-containing tissue.

In an attempt to produce an autoimmune kidney lesion in the sheep, glomerular basement membrane (GBM), prepared from kidneys of man, monkey, rat, rabbit, or dog, incorporated into Freund's complete adjuvant, was injected by various routes into adult female sheep every 14 days. Twelve sheep received 480 to 1,419 mg. of GBM in 3 to 6 injections. Each sheep received GBM prepared from a single species. All 12 sheep died in uremia 38 to 90 days after injections began. All sheep had heavy proteinuria and elevation of the blood urea nitrogen to between 350 and 450 mg. per cent. Grossly the kidneys were swollen, gray brown, and covered with petechiae. Microscopically, all kidneys showed severe extracapillary glomerulonephritis characterized by: marked fibroepithelial proliferation (crescents) obliterating Bowman's space, casts and blood in tubules, various retrogressive changes in tubules, atrophy and dilatation of tubules, increased interstitial connective tissue, and varying amounts of interstitial round cell infiltration.

Seven control sheep were injected with Freund's adjuvant alone every 14 days; all received 6 to 9 injections. They were sacrificed 100 to 118 days after the first injection. One sheep had mild proteinuria and glomerular damage. The remaining 6 sheep were essentially normal.

In conclusion, kidney damage in sheep receiving GBM presumably resulted by an autoimmune mechanism initiated by injection of a heterologous kidney antigen incorporated in Freund's adjuvant. This regular and uniformly successful production of a fulminating, fatal experimental autoimmune nephritis in sheep, with renal lesions remarkably similar to those of various stages of human glomerulonephritis, lends support to the autoimmune theory of human glomerulonephritis.

116. The isocitric dehydrogenase test in the differential diagnosis between medical and surgical jaundice

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Differentiation between medical and surgical jaundice is difficult, especially in cases with poor history, lack of physical findings, and equivocal laboratory tests. The transaminases

which originally were considered helpful in this differentiation have been found abnormally high in some types of surgical as well as medical jaundice. We therefore evaluated the recently introduced isocitric dehydrogenase test in cases of medical and surgical jaundice.

Eighty cases of acute hepatitis and 15 of surgical jaundice were studied. The results were as follows:

The isocitric dehydrogenase average in acute hepatitis was 1,149, with a serum glutamic-oxalacetic transaminase and serum glutamic-pyruvic transaminase average of 522 and 697, respectively. In the surgical cases, the isocitric dehydrogenase average was 595 and the serum glutamic-oxalacetic transaminase and serum glutamic-pyruvic transaminase were 151 and 77, respectively.

If 3 cases of malignant obstruction with liver metastases, which have higher enzyme values, are excluded from the surgical jaundice cases, the isocitric dehydrogenase average drops to 380 and the serum glutamic-oxalacetic transaminase and serum glutamic-pyruvic transaminase to 90 and 66, respectively.

A breakdown by duration of illness showed the isocitric dehydrogenase average to be 2,071 in the first week, 1,378 in the second, and 1,213 in the third week. In 5 cases which were observed longer, the average values receded from 1,213 to 1,186, 965, 600, and to 165. In patients whose symptoms persisted, a high isocitric dehydrogenase was found for several weeks.

Of particular interest was the finding that in hepatitis with intrahepatic obstruction as evidenced by absence of urobilinogen from the urine, the isocitric dehydrogenase values were very high—average 2,618 in ten cases. This test may, therefore, prove helpful in the differentiation between medical and surgical jaundice.

117. Alterations in human cerebral hemodynamics using external isotope counting

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Measurement of human cerebral blood flow has been essentially limited to the nitrous oxide method. Sapirstein has measured cerebral blood flow using 4-iodo^{131} antipyrine (4IAP) and K^{42}. This study describes a method using 4IAP and rubidium^{86} chloride (RbCl) to measure human cerebral blood flow. 4IAP crosses the blood-brain barrier with ease, but there is no readily exchangeable cerebral pool for Rb^{86} or K^{42}. After a rapid intravenous injection 4IAP is distributed to a sufficiently large body and brain pool, the cephalic washout rate (brain, head, and neck) is slow enough to permit semilogarithmic extrapolation to maximum cephalic content at zero time. Rb^{86} has been demonstrated to be distributed to a sufficiently large body pool in one circulation after a rapid intravenous injection so that the organ contents remain stable for a considerable period of time, and thus are proportional to the distribution of the functional perfusion to the organs. Since the brain pool for Rb^{86} is small, the non-cerebral cephalic content can be determined with this tracer, The difference in the cephalic contents between 4IAP and Rb^{86}, corrected for the cerebral Rb pool and recirculated Rb from the brain, is that fraction of the cardiac output distributed to the brain. In the determination of the cephalic content of each of the isotopes a gamma ray–sensitive well counter sufficiently large to contain a human head was used.

Cardiac output was measured by the indicator dilution principle using cardiogreen by direct arterial sampling. In 16 subjects the cardiac index was 3.13 ± 0.53 L. per square meter per minute, and total cerebral flow was 751 ± 179 ml. per minute. Duplicate resting values for cerebral flow averaged 763 ± 75 ml. per minute and 766 ± 81 ml. per minute with a coefficient of correlation of 0.96. Hyperventilation reduced the mean arterial pCO_2 to 20 mm. Hg, increased the cardiac output 1.16 L. per minute, and reduced the cerebral blood flow to

455 ± 65 ml. per minute. Inhalation of 5 per cent CO_2 increased the arterial pCO_2 5 mm. Hg, increased cardiac output 440 ml. per minute, and increased cerebral blood flow to 950 ± 73 ml. per minute. These results demonstrate dynamic responses of the total cerebral circulation rather than steady state measurements per unit mass of brain as reported by the nitrous oxide method.

118. The diagnosis of acute myocardial infarction on the basis of heat-stable serum lactic dehydrogenase

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Introduced by Ivan D. Frantz, M.D.

Serum lactic dehydrogenase (LDH) activity usually increases sharply after an acute myocardial infarction. However, excesses of LDH arising from the liver, as in congestive heart failure, or from skeletal muscle postoperatively frequently make the diagnosis equivocal.

We have demonstrated that cardiac LDH is relatively heat stable and that liver LDH is remarkably heat labile. These properties may be helpful in determining which tissue is releasing excessive amounts of LDH.

Liver homogenates which are incubated at 65° C. for 30 minutes lose 99.5 per cent of their LDH activity, whereas similar incubation of heart homogenates inactivates only 47 per cent of the LDH activity.

The thermostability of serum LDH from patients with acute myocardial infarction, infectious hepatitis, congestive heart failure, and recent surgical procedures was also studied. At the peak elevation of serum LDH in patients with myocardial infarction, 43 per cent of the total serum LDH activity was lost after incubation at 65° C. for 30 minutes. The remaining 57 per cent of the LDH is considered "heat stable." The activity of this heat-stable LDH averaged four times greater than the upper limit of the normal level of heat-stable serum LDH. In contrast, 87 to 93 per cent of the total serum LDH activity from patients with acute congestive heart failure or hepatitis was lost by incubation at 65° C. In these patients the remaining heat-stable LDH was present at the level found in normal subjects. In postoperative patients 82 to 89 per cent of the total serum LDH activity was lost by incubation at 65° C. for 30 minutes. The remaining heat-stable LDH activity in this group was the same as that found in normal subjects.

In summary, heat-stable LDH appears to arise mainly from heart muscle. Hence, determination of the heat-stable serum LDH activity permits the diagnosis of acute myocardial infarction during the postoperative period and in patients with congestive heart failure or infectious hepatitis.

119. Hematologic toxicity of a chloramphenicol analogue

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Early erythropoietic depression due to chloramphenicol can be detected by changes in serum iron, prolonged plasma clearance of radioactive iron, and delayed reappearance of radioactive iron in circulating erythrocytes. These changes occur 1 to 2 weeks prior to the fall in hematocrit and can be used to recognize erythropoietic depression before irreversible damage to the bone marrow occurs. Since the nitrobenzene moiety of chloramphenicol has been implicated as a possible cause of the toxicity, a study of choramphenicol analogues, using the above parameters, was undertaken. An increased incidence of hematologic toxicity occurred when a CH_3SO_2 group replaced the NO_2 group in the chloramphenicol structure. The present report deals with another analogue of chloramphenicol in which the NO_2 group is replaced by a methylmercapto group (CH_3S). The spectrum of antibacterial action remained essentially unchanged.

This drug was administered orally to 14 patients with a chronic disease process in a daily dose of 2 Gm. for periods up to 21 days. Complete blood counts were made and serum iron and iron-binding globulin were measured weekly. Plasma clearance of Fe^{59} ($T\frac{1}{2}$) and its reappearance in circulating erythrocytes were determined in all patients during the study period. Six patients showed a fall in hematocrit of 5 per cent or more with a prolonged plasma clearance of Fe^{59} and a delayed reappearance in the red cells. The serum iron increased from an average control value of 87 μg per cent to 192 μg per cent. The per cent saturation of iron-binding globulin rose from an average control value of 41 per cent to 66 per cent. As in previous studies, these changes preceded the fall in hematocrit by 1 to 2 weeks. Four of the 6 patients developed leukopenia, but in no instance was there a thrombocytopenia. All values returned to normal levels within 4 weeks of discontinuation of the drug.

These studies continue to implicate the para-position of the nitrobenzene moiety in the structural configuration of chloramphenicol and its analogues as a factor in hematologic toxicity and confirms the value of rises in serum iron and iron-binding globulin saturation as an index of erythropoietic depression.

120. Man's sources of plasma cholesterol

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Heretofore there have been no accurate data on the extent to which the cholesterol in plasma may be derived from absorption of dietary cholesterol or from synthesis by liver and/or extrahepatic tissues. Administration of a cholesterol-rich diet suppresses and virtually eliminates hepatic cholesterol synthesis in all species studied. If the dietary cholesterol is labeled (cholesterol-4-C^{14}) with a known and constant specific radioactivity (counts per minute per milligram cholesterol), as hepatic cholesterol synthesis is replaced by absorption of dietary cholesterol, the plasma cholesterol specific radioactivity progressively rises and levels off at some maximum value. At this time the ratio of plasma cholesterol specific activity to dietary cholesterol specific activity is a fractional value that represents both the proportion of plasma cholesterol that came from the diet and the approximate proportion that could come from liver if diet supplied none. In either situation the remaining proportion of plasma cholesterol must come from synthesis by extrahepatic tissues. In dogs, upon the continued administration of a cholesterol-rich egg yolk diet, about 90 per cent or more of plasma cholesterol is supplied by dietary cholesterol. In studies on man, 5 groups of subjects have ingested constant known dosages of cholesterol-4-C^{14} in egg yolk (2 doses per day providing 20 Gm. of dried yolk and either 0.5, 0.8, 1.4, 2.4, or 4.4 Gm. of cholesterol) for 8 weeks or longer with radioassay of weekly plasma cholesterol samples. To date the studies all show that adult human subjects can obtain about one fourth to one third of their plasma cholesterol by absorption of dietary cholesterol if they ingest approximately 1 Gm. of cholesterol per day. The plasma cholesterol radioactivity curves of subjects on 1.4, 2.4, and 10.4 Gm. per day reach a maximum in 3 to 5 weeks and appear to level off at a constant plateau value thereafter. The uptake curves of subjects on 0.5 and 0.8 Gm. per day are considerably more erratic and slow, although even a dosage of 0.5 Gm. cholesterol per day eventually supplies nearly as large a proportion of plasma cholesterol as do higher dosages.

121. The relationship of the juxtaglomerular apparatus to sodium retention in experimental nephrosis

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The juxtaglomerular apparatus, with its associated renin, appears to have a relationship to the renal handling of sodium. There is a strong possibility that most edema states are as-

sociated with a hypersecretion of renin by the juxtaglomerular apparatus. Renin is known to augment the secretion of aldosterone. To examine this relationship, we studied the juxtaglomerular cells in experimental nephrosis. A number of rats were given a standard regimen of aminonucleoside. After 18 days the rats were killed, the amount of ascites was measured, and kidney tissue was obtained for specific staining and quantitation of juxtaglomerular granules. The rats were divided into 2 groups. Each of the 32 rats of one group had between 4 and 30 ml. of ascitic fluid, the 28 rats of the other group had between 0 and 1 ml. The group with considerable ascites had an average juxtaglomerular index of 33 (S. D. ± 9). The group with minimal ascites had an average juxtaglomerular index of 21 (S. D. ± 6). This difference is highly significant ($p < 0.0000001$). For each rat of the two groups the juxtaglomerular index was plotted on a scattergram in relation to the amount of ascites. The coefficient of correlation between these two variables was +0.72. This is a highly significant degree of correlation ($p < 0.000001$). Serum creatinine at the end of the experiment averaged 0.72 mg. per cent for the rats with considerable ascites and 0.73 mg. per cent for the rats with minimal ascites, suggesting that glomerular filtration was equivalent in the two groups. None of the rats in either group had a significant degree of nitrogen retention.

Hypergranularity of the juxtaglomerular cells indicates an increase in the rate of secretion. Moreover, juxtaglomerular hypergranularity in the rat is invariably associated with an increased amount of extractable renin. The data here showed that the amount of sodium retention varies directly with the degree of juxtaglomerular granularity. This strongly suggests that sodium retention occurs concomitantly with juxtaglomerular hypersecretion. This observation is consistent with the hypothesis that the juxtaglomerular apparatus has an important effect on the renal retention of sodium.

122. The two chambered right ventricle: Report on seven patients

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Introduced by Alexis F. Hartmann, Sr., M.D.

The division of the right ventricle into two chambers by hypertrophic muscular bands is a very unusual congenital cardiac malformation which may be mistaken for tetralogy of Fallot or an interventricular septal defect, but cardiac catheterization, and especially selective angiocardiography, will establish the correct diagnosis. The muscular bands divide the right ventricle into a high pressure chamber immediately distal to the tricuspid valve, and a low pressure chamber bounded by these bands and the pulmonic valves. This malformation may occur as the only defect but it is often associated with an interventricular septal defect which may be located at the junction, above, or below the plane of the hypertrophied muscular bands.

This is a report about 7 patients, aged 2 to 10 years, with this anomaly, in whom the diagnosis was established by cardiac catheterization and selective angiocardiography. All patients had a loud left basilar systolic murmur and thrill, and the second sound in this area was diminished in intensity. The electrocardiogram was within normal limits in 4 patients, 1 showed right ventricular enlargement, 1 showed findings suggestive of this, and 1 showed incomplete right bundle branch block.

Cardiac catheterization revealed pressures in the high pressure chamber of 55/0 to 133/0 mm. Hg, while the pressures in the distal chamber were 24/0 to 49/0 mm. Hg. Five of the 7 patients had an associated interventricular septal defect and the flow was left to right.

Three of the patients were operated upon successfully with the aid of extracorporeal support. The ventricular septal defect was repaired and the hypertrophied muscular bands were resected.

123. Recall of type-specific antibodies in man by injections of streptococcal cell walls

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Because of the toxicity of group A streptococcal products, attempts at human vaccination have been confined to the administration of small doses of preparations containing the type-specific virulence factor, M protein. To date such small doses have not resulted in a consistent type-specific primary immune response. The antigenicity of M protein in these doses in man, therefore, has been questioned. During 4 years of a controlled study of streptococcal pharyngitis in children, type-specific antibody has disappeared from the blood of approximately half of the patients who initially developed an immune response. Advantage was taken of this situation to study the antigenicity of T12 and T5 cell walls by means of the recall phenomenon.

Ten children were selected who had formerly developed unmistakable T12 antibody responses following T12 pharyngitis but in whom the titer of this antibody subsequently disappeared (7 patients), or diminished considerably (3 patients), during the following 2 years. T12 antibody was recalled in all of these patients after doses of 4 to 44 mg. cell wall nitrogen given in 3 or 4 injections, subcutaneously or intradermally, at weekly intervals.

Preparations of T12 or T5 cell walls were administered to an additional 10 patients whose antecedent T12 or T5 infections had been followed by little or no demonstrable type-specific antibody. Homologous type antibody was boosted in 6 patients, 5 of whom had had probable traces present before the injections were given. An additional 14 patients were selected who had no previously documented T12 infections. Following injection of cell walls T12 antibodies were recalled in 7 patients. These included all 5 who had had traces of antibody at some time prior to immunization.

Therefore, in all instances in which type-specific antibodies had been present in definite or trace amounts at some time prior to immunization, injections of well-tolerated, small amounts of homologous type streptococcal cell walls resulted in a secondary immune response. Although these responses were relatively weak, and with a few exceptions persisted less than 6 months, the titers were comparable to those observed after the original, mild natural infection.

124. Studies of the immune response to jackbean urease in patients with hepatic insufficiency

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Introduced by L. J. Roth, M.D.

Four patients with hepatic insufficiency, 3 of whom had portal systemic surgical shunts, have been immunized with jackbean urease. Antibody production in these patients has been followed by hemagglutination titers using formalinized human erythrocytes sensitized to urease. Immunity to this enzyme has been produced by weekly injections of 150 μg of protein (20 Sumner units of urease activity). Patients treated by this schedule have developed detectable titers in 7 to 13 days. Six weekly injections have resulted in titers ranging from 1:100,000 to 1:500,000. Throughout the course of the injections the patients have exhibited signs of prehepatic coma 4 or 5 days after the second or subsequent injections. These episodes correspond to the time of antigen disappearance in an anamnestic response. Daily blood ammonia levels and antibody titers are now being investigated following antigenic challenge. Immunized animals exhibit a 50 per cent decrease in C^{14} urea metabolism in vivo and simultaneously have 40 to 60 per cent less in vitro urease activity in the gastrointestinal contents. Urease immunity thereby duplicates the in vivo and in vitro effects on urea hydrolysis we observed in rats following intake of antibacterial agents at 100 ppm in the diet. Both urease immunity and dietary antibacterial agents have produced growth increases in animals and

birds of similar magnitude. Antibacterial agents at the above dosage have not been shown by others to produce qualitative or quantitative changes in the pre-existing bacterial flora. Passive transfer of antisera has lowered blood ammonia levels in normal rabbits and protected them against lethal doses of urease. Acute LD_{50} values in rats and mice have been found to be about 375 μg urease per kilogram of body weight. However, in the immune animal, daily injections of 375 μg per kilogram of urease for 60 days produced no gross evidence of pathologic damage. Antibody to the enzyme has been found in the feces and urine of immunized animals. The preliminary findings suggest that patients with liver disease may derive benefit from a physiologic mechanism of antibody formation which inhibits a bacterial-ammonia producing system.

125. The effect of nicotinic acid and nicotinamide on pyridine nucleotide levels and glucose and lipid metabolism

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Introduced by J. F. Mueller, M.D.

Nicotinic acid in large doses has been used therapeutically as a hypocholesterolemic agent. There are isolated clinical reports of abnormalities in glucose metabolism during the course of nicotinic acid treatment.

In an effort to establish a clear association between the alterations in glucose and lipid metabolism induced by large doses of nicotinic acid, it appeared desirable to study pyridine nucleotide levels during the oral administration of the acid or nicotinamide to 7 hypercholesterolemic patients.

After an initial control period, either nicotinic acid or nicotinamide was administered orally at the dose of 3 Gm. per day for 1 month. The drugs were then discontinued until the parameters studied approached the control levels. The alternate drug was then administered orally at the same dose for 1 month.

While the patients were taking nicotinic acid, the following changes, compared to control values, were repeatedly noted: the diphosphopyridine nucleotide (DPN) concentration in blood rose to 5 times the control levels. The blood contents of triphosphopyridine nucleotide (TPN) remained unchanged. The blood glucose disappearance rate following 0.5 Gm. glucose per kilogram body weight, administered intravenously, was slower. The hypoglycemic response to 0.1 U. per kilogram weight of glucagon-free insulin intravenously showed no alterations; however, the intravenous administration of 1 Gm. of tolbutamide produced a smaller percentage fall of glycemia. Total blood ketone levels and their response to insulin hypoglycemia were increased. Plasma-free fatty acid levels rose. Plasma cholesterol, phospholipids, and glycerides decreased.

The administration of nicotinamide produced no alterations in any of the parameters studied.

These findings would suggest that, in human beings, nicotinic acid, as compared to nicotinamide, produces the foregoing alterations in glucose and lipid metabolism by inducing marked increases in diphosphopyridine nucleotide levels. These pronounced changes in the DPN/TPN ratio could produce, through the regulatory role of pyridine nucleotides in glucose and lipid intermediary metabolism, alterations leading to lipid mobilization, lipolysis, shifts in glucose oxidative pathways, and decreased cholesterol synthesis.

126. Sulfur^{35} as a label for human platelet survival studies

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Introduced by Henry E. Hamilton, M.D.

Knowledge of human platelet survival in pathologic states is still incomplete. Intravenous sodium S^{35} sulfate was used to avoid repeated handling of platelets and to provide a firm

label by its metabolic incorporation into the megakaryocytic sulfated–mucopolysaccharides. Rapid excretion of unincorporated isotope prevented its reutilization. Two methods of intravenous administration were used: either one single dose or multiple equal daily doses. After a single dose plasma levels declined rapidly, and 58 to 70 per cent was recovered in the 24 hour urine samples. Total urine S^{35} recovery was 80 per cent in 5 to 9 days. Semilog plots of urinary excretion and plasma levels both indicated a multiple phase removal. With multiple equal doses, plasma samples obtained from patients just before each daily dose showed a gradual rise in radioactivity. Total urine S^{35} recovery was 83 to 91 per cent. Urinary radioactivity fell rapidly following cessation of daily doses.

Before platelet radioassay, an aliquot of platelet suspension was counted by direct phase microscopy determining the number of platelets in each sample. Hemocytometer chamber count excluded the presence of leukocytes.

All platelet, plasma, and urine samples were assayed in liquid organic scintillation mixture for beta counting.

A single-dose method was used in 1 patient with chronic granulocytic leukemia. A multiple daily dose method was used in 2 patients with chronic granulocytic leukemia, 2 patients with polycythemia vera, and 1 patient with no hematopoietic disease. Platelet survival was estimated to be 10 days in all these patients, except in 1 man with chronic granulocytic leukemia and pronounced splenomegaly, whose shortened platelet survival time was 7 days.

In vivo labeling of platelets with S^{35} has specific advantages: avoidance of repeated handling of platelets, metabolic megakaryocytic incorporation providing firm platelet label which eliminates elution, rapid excretion of unincorporated isotope reducing radiation exposure and preventing reutilization, and physical half life of 87.1 days. Data concerning urinary excretion rates and plasma levels of S^{35} are presented. Relative ease of sample preparation for beta liquid scintillation counting eliminates some procedures which compound the error of techniques used in other studies.

127. The comparative method of determining the clinical efficacy of an antimicrobial drug

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The fact that the apparent clinical efficacy of antimicrobial agents will vary inversely with the severity of the infections being treated may explain the wide divergence of results of clinical trials with new drugs.

The present study describes a method of determining the efficacy of an antimicrobial drug in which the patients with comparable infections are chosen at random for treatment with one of several drugs. Data are analyzed to determine the similarity of the groups treated with each agent. The results of treatment in each group are analyzed by all available criteria.

The method is illustrated by a study of the relative activity of furaltadone and novobiocin in patients with severe staphylococcal infections. In spite of efforts to exclude it, bias is shown to have crept into patient selection. Limitations are clearly shown of serial blood and wound cultures, and of temperature curves, as indicators of drug response.

These data support the conclusion that furaltadone is neither as effective nor as ineffective as previous studies have indicated but, perhaps more important, they emphasize and delineate some of the difficulties inherent in the critical evaluation of new drugs

128. The application of galactose titration to the study of "lobular perfusion" in normal and cirrhotic livers

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Application of saturation methods to the study of nephron distributions is well known. An attempt is made here to develop an analogous saturation method suitable for the study of the distribution of "lobular perfusion" (blood flow per unit functioning mass) in the liver.

The method employs the constant galactose infusion titration technique we have described previously (J. LAB. & CLIN. MED. 56: 920, 1960). In each subject, measurement of total body galactose removal (R) is made at 6 to 9 successively higher peripheral blood concentration equilibria (Peq), such that 2 Peq's below 30 mg. per 100 ml. be obtained and that the highest Peq result in a hepatic load (Ld) in excess of the total body removal maximum (R_m). Hepatic blood flow (F) and extrahepatic galactose removal (E_r) are estimated from the 2 low equilibria: $F = \frac{R_2 - R_1}{Peq_2 - Peq_1}$; $L_{r_1} = F \times Peq_1$; and $E_r = R_1 - L_{r_1}$, L_r being hepatic galactose removal. For the other equilibria: $Ld = Peq \times F$ and $L_r = R - E_r$. The hepatic removal maximum (L_m) $= R_m - E_r$. The titration curve is constructed by plotting L_r/L_m ratios on the ordinate and Ld/L_m ratios on the abscissa. With such coordinates, it is possible to pool data from different subjects despite individual differences in F and L_m.

The pooled normal titration curve constructed from 65 equilibria in 10 subjects without liver disease shows a small amount of splay. Although most functional units appear to saturate near a Ld/L_m ratio of 1.0, a few per cent saturate at ratios as low as 0.7 and as high as 1.7. This suggests a relatively narrow, homogenous "lobular perfusion" distribution in the normal liver.

The pooled titration curve in Laennec's cirrhosis constructed from 51 equilibria in 8 patients shows considerably more splay, many functional units saturating at abnormal Ld/L_m ratios, indicating a greater than normal distribution in the amount of perfusion per unit functional mass. Since F is proportionately more reduced than L_m, it is suggested that the abnormal "lobular perfusion" found in cirrhosis results primarily from alteration of the blood supply rather than of the parenchymal mass.

129. The effect of oral potassium salts on functional and organic T waves

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The need for an expedient procedure to differentiate clearly functional from organic T waves in the field of clinical electrocardiography has long been recognized.

Based upon our previous experiences with the consistent ameliorating effect of oral potassium salts on functional precordial T wave inversion, as seen in the "juvenile pattern," 10 Gm. of oral potassium salts have been administered to 108 patients with clinical and electrocardiographic evidence of the patterns listed below.

Acute transitory transmural ischemia	20
Acute subendocardial ischemia	15
Acute posterior wall infarction	15
Acute anterior wall infarction	15
Healed posterior wall infarction	5

Healed anterior wall infarction	5
Left bundle-branch block	10
Left ventricular preponderance	10
Digitalis effect	10
Pericarditis, subacute	2
Myxedema	1

Only 4 patients with left ventricular preponderance and 2 patients with acute but transitory transmural ischemia showed improvement, but no normalization of the T wave inversion, and specifically, the improvement tended to involve only the "fringe areas" of the inverted T waves. All other instances of the organic T wave inversion, irrespective of the etiology, showed no change in polarity or configuration.

Thus, it appears that the oral administration of potassium salts, in large dosage, is of considerable clinical value in differentiating electrocardiographic T wave changes of functional origin (anxiety induced) from those on an organic basis. The procedure has been well tolerated and without untoward reactions.

130. An anomaly of granulocytes and platelets: A newly established hereditary entity

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In 1909, May described an anomaly of granulocytes characterized by an area of basophilic cytoplasm in otherwise normal cells and in giant platelets. In 1945, Hegglin reported similar findings in a man and his 2 sons. We found no other reports.

This report presents histochemical studies of the anomaly occurring in 13 members of three generations of one family and establishes it as a hereditary entity.

Peripheral blood smears were dried in air and stained with Wright's stain. Segmented granulocytes had an area of blue cytoplasm without granulation, about 1 by 3 μ in diameter, usually fusiform or crescentic with well-defined borders, and located anywhere within the cytoplasm. Methyl green pyronin Y stained the area pink, indicating a large component of ribose nucleic acid. After incubating the leukocytes with ribonuclease, pyronin Y staining was negative. Feulgen stain for desoxyribose nucleic acid was negative. Periodic acid–Schiff stain for adjacent glycol groups was negative. Electron microscopy revealed spaces without granules in the cytoplasm.

Platelets were found singly and in clumps. Most were about 6 μ in diameter and round, some were as large as 15 μ and elliptical. Most were well granulated; some had only a few granules scattered over a light blue hyalomere. Others had only a few granules clumped in the center and surrounded by a pale hyalomere from which many fine projections extended.

The anomaly affected 4 women and 9 men, from 4 to 73 years of age, in three generations. There was no skipping in the phenotypical representation of this anomaly, indicating that it is inherited as a simple dominant. Blood typing (ABO, MN, D, D^u), in all cases was compatible with presumed parenthood. Hemoglobin values and white blood cell counts were normal. There were no clinical manifestations associated with the anomaly.

This report presents 13 cases of an anomaly of granulocytes and platelets, not described before in America. The histochemical nature of the cytoplasmic anomaly in these granulocytes was established and a mode of genetic transmission was determined.

131. The effect of intravenous estrogen on tissues and capillaries as measured by coagulation tests, intradermal hyaluronidase, hyaluronidase serum inhibitor, and pulmonary diffusion studies

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Experiments in animals and humans indicate that estrogens increase the mucopolysaccharide content of ground substance. Recently, intravenous estrogen, Premarin, has been used to improve hemostasis. The basis for its reported effectiveness is uncertain.

Double blind studies were performed on human subjects with the use of coagulation tests, intradermal hyaluronidase spread, hyaluronidase serum inhibitor levels, and pulmonary diffusion capacity before and after intravenous injection of 40 mg. of Premarin or placebo. In 51 subjects Ivy bleeding time decreased from a mean of 6.1 to 4.6 minutes after the estrogen ($p = 0.01$) compared with 5.8 to 5.6 minutes after the placebo (34 cases). Factor V values increased in 20 of 47 subjects receiving the estrogen and in 2 of 35 after the placebo. The mean increase of 8.27 per cent ($p = 0.001$) occurred within 1½ hours after injection. Clotting time, thromboplastin generation, factor VII, and antithrombin assays remained unchanged.

Intradermal hyaluronidase wheals were injected 30 minutes after administration of estrogen or placebo and the wheals were observed until disappearance. The mean "disappearance time" in 13 men was 50 minutes with estrogen, 33 minutes with placebo ($p = 0.001$). In women no significant differences were observed between estrogen (53 minutes), and placebo (51 minutes).

Serum normally contains inhibitors to hyaluronidase. A turbidimetric method employing testicular hyaluronidase, umbilical cord hyaluronic acid, and serum was used to study the effect of estrogen on these inhibitors. There was a mean inhibitor increase of 19 per cent in 17 of the 18 subjects ($P > 0.01$) one hour after the hormone administration.

Finally, the responsiveness of the pulmonary capillary bed to estrogen was studied. Diffusion capacity was measured with the single breath CO method, 24 hours before and 15 minutes, 45 minutes, 2 hours, and 24 hours after administration of estrogen. Twenty-four subjects were tested. The 40 mg. dosage produced a statistically significant drop in the diffusion capacity in both men and women ($p = 0.001$). The capacity in control subjects remained constant.

These various experiments suggest that estrogen increases the ground substance of the capillary bed. Its presumed effect on the control of bleeding may be related to this response.

132. Effect of isotonic saline upon metabolism of intravenous hexoses in normal dogs

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Electrolytes are known to affect carbohydrate metabolism. This is the first report of our investigations concerning the effect of different electrolytes upon the metabolism of parenterally administered carbohydrates under various conditions. Approximately 100 normal female dogs, anesthetized with Nembutal in the postabsorptive state, were infused via a constant injection pump with 10 per cent solutions of 7 combinations of hexoses

(glucose, fructose, and galactose) in distilled water *or* in isotonic saline at a rate of 1.0 Gm. per kilogram per hour for 5 hours. Blood was drawn at hourly intervals for various determinations and biopsies of liver, gastrocnemius and cardiac muscle were obtained at the end of the infusion for determination of glycogen concentrations. Of the various parameters studied in these normal dogs only liver glycogen was different in the two series. The liver glycogen of dogs given glucose in isotonic saline was only 63 per cent of that found with the administration of glucose in water. In contrast, fructose in isotonic saline resulted in liver glycogen values of 153 per cent of those with fructose in water. Saline had no significant effect on the liver glycogen of dogs given 5 additional combinations of hexoses. The *lowest* values of liver glycogen were found with glucose in saline, fructose in water, or glucose in water; the *highest* were associated with invert sugar in water, fructose in saline, or invert sugar in saline.

The concentrations of liver glycogen increased in the following order: glucose in saline, fructose in water, glucose in water, invert sugar in water, fructose in saline, and invert sugar in saline. The therapeutic significance of the reported "glycosurias" of these commonly used hexoses in relation to our data of liver glycogen concentrations will be discussed.

133. Altered globin formation in human megaloblasts incubated with nucleoproteins derived from sickle cell anemia

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Abnormal globin production in megaloblastic marrow following incubation with nucleoproteins derived from sickle cell anemia marrow was reported in a previous communication. The abnormal globin formation was demonstrated by electrophoretic procedures, carboxymethylcellulose chromatography, and peptide "fingerprint" analysis. This report deals with further observations on this phenomenon.

Evidence that the abnormal globin contains newly incorporated amino acids was obtained by incubating megaloblastic marrow with nucleoproteins derived from sickle marrow and with C^{14} leucine. The hemoglobins were separated by carboxymethylcellulose chromatography and the radioactivity in each fraction was compared with the optical density at 580 mμ. A peak of radioactivity was found to coincide with an abnormal hemoglobin. Preliminary data indicate that abnormal globin production may occur in reticulocytes also. Peripheral blood from patients with sickle cell anemia containing 25 to 30 per cent reticulocytes was incubated with nucleoproteins obtained from normal marrow. Altered globin formation was demonstrable by carboxymethylcellulose chromatography. The elution behavior of this component was similar to but not identical with hemoglobin A.

The nucleoproteins used in these experiments were highly viscous material containing 5 to 10 per cent ribonucleic acid, 5 to 10 per cent protein, and 80 to 90 per cent deoxyribonucleic acid. To determine which of these components was the active material, enzymatic degradation of each component was carried out and the effect of the remaining material on globin production by megaloblastic marrow determined. Abnormal globin formation occurred when the nucleoprotein complex was incubated with either deoxyribonuclease or trypsin. No abnormal protein formation has been observed following incubation with ribonuclease. An unexpected observation was that, following enzymatic action, abnormal globin production could occasionally be obtained even though the original material was ineffective. Thus, in 3 experiments the abnormal globin was formed only after the nucleoprotein complex was exposed to deoxyribonuclease. This suggests that deoxyribonucleic acid may compete with ribonucleic acid for receptor sites, or that the former may be complexed with the latter acid so as to interfere with the biologic

activity of ribonucleic acid, and that deoxyribonuclease releases ribonucleic acid from this complex. Possibly, polynucleotides formed by the enzymatic action of deoxyribonuclease effect the abnormal peptide formation. Further studies on this observation are in progress.

134. Myocardial involvement in muscular dystrophy

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In muscular dystrophy both myocardial and skeletal muscle abnormalities may be found. Involvement of the myocardium in this condition constitutes a specific cardiomyopathy. Clinical evidence of myocardial involvement includes labile tachycardia, congestive heart failure, various arrhythmias, and cardiomegaly. In many of these patients electrocardiographic abnormalities are also present. Histopathologic changes similar to those encountered in skeletal muscle are usually present. Cardiac metabolism was studied in 11 patients with muscular dystrophy (10 Duchenne and 1 facioscapulohumeral) by means of coronary sinus catheterization. Simultaneous arterial and coronary vein bloods were analyzed for oxygen, glucose, pyruvate, lactate, inorganic phosphate, malic dehydrogenase, and aldolase. In 2 patients determinations were done at rest and during exercise. Glucose was extracted by the heart in all; pyruvate was utilized in 7 and released in 2 subjects. Lactate was extracted by the heart in all but 1 patient, in whom it was released. Aldolase was released from heart muscle in 3 patients. Inorganic phosphate concentration in blood was elevated in all patients, but it was neither released nor extracted by the heart. Malic dehydrogenase activity in the plasma was above normal in 50 per cent of the patients. Differences in the oxidation-reduction potential between arterial and coronary vein blood were positive, suggesting glycolysis. This was accompanied by an increase in the myocardial extraction of glucose, suggesting an increase in glycolysis rather than respiration. Glucose oxygen extraction ratios were less than 100 per cent in all patients studied, indicating myocardial breakdown of substrates other than glucose. Apparently, aerobic glycolysis occurred because sufficient oxygen was present to account for all glucose extracted. As serum inorganic phosphate increased, myocardial glucose extraction rose in a linear relationship, suggesting a causal relationship between glycolysis and the increase in inorganic phosphate. An increase in inorganic phosphate, the elevation in cardiac output, and the presence of glycolysis suggest uncoupling of oxidative phosphorylation. Glycolysis may represent an important source of energy in the hearts of patients with muscular dystrophy.

135. Further studies of adrenocortical function in bronchogenic carcinoma

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Previous investigation suggests increased adrenocortical activity in lung cancer patients without Cushing's syndrome. This report represents a more comprehensive appraisal which includes correlation of adrenocortical function with disease progression and malignant cell type. Sixty-five patients with bronchogenic carcinoma were studied at various stages; interval to death ranged from 1 to 64 weeks.

Plasma unconjugated 17-hydroxycorticosteroid levels (17-OHCS), 7 to 9 A.M., correlated inversely with interval to death. Patients living more than 30 weeks usually had normal levels. Six to 30 week levels were elevated, mean 20 μg per cent; 5 week to death levels manifested further increase. Urinary total 17-OHCS tended to show similar correlation, but with greater scatter, including several low values, from 10 weeks to death.

No correlation among plasma 17-OHCS and cell type was observed. Urinary total 17-OHCS were higher in patients with oat cells who died within 5 weeks, but elevated levels (> control mean + 2 S. D.) were found occasionally in patients with other cell types. The incidence of elevated urinary total 17-OHCS was 57 per cent in patients with brain involvement; high levels were also observed in the absence of discernible brain dysfunction. The over-all incidence of elevated urinary total 17-OHCS was 29 per cent, substantiating increased adrenocortical secretion.

Although significant correlation was found between plasma 17-OHCS and urinary total 17-OHCS, r 0.51, there was a disparity, with plasma values being relatively higher. Altered hepatic cortisol metabolism is suggested because urinary unconjugated 17-OHCS manifested higher correlation with plasma, r 0.92, and the ratio of urinary unconjugated to total doubled in patients with advanced disease with elevated plasma 17-OHCS. There was significant correlation between urinary total 17-OHCS and 17-ketogenic steroids, r 0.62, implying that cortisol was not metabolized preferentially via C-20 reduction.

Forty-two patients were tested with ACTH. The mean rise in plasma 17-OHCS remained unchanged as death approached, but urinary total 17-OHCS response decreased, suggesting diminishing adrenal reserve in conjunction with the above-mentioned metabolic defect. Two patients in terminal condition showed markedly decreased ACTH responses. ACTH hyperresponsiveness was observed in 9 patients.

Correlation of postmortem adrenals (40 patients) and antemortem steroid values yielded interesting findings. Of 11 patients with elevated urinary 17-OHCS, the adrenals of 4 showed hyperplasia, whereas 2 revealed almost complete metastatic destruction. Of 5 patients with ACTH hyperresponsiveness, 3 demonstrated hyperplasia and 1 demonstrated metastatic destruction.

136. Clinical and immunochemical evidence for host resistance to tumor

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In the natural history of patients with cancer, one observes spontaneous regression, postsurgical regression, regression of lesions distant from irradiated parent tumors, increased growth rate of tumors following both cortisone therapy and the breakdown of local tissue resistance from excessive irradiation. Sir Gordon Taylor reported case histories which documented increased growth rate of tumors with severe stress. Engell estimated the number of circulating cancer cells in patients with malignancy. He followed these patients for 5 to 10 years to find no correlation between the number of circulating cells and metastatic nodules. These clinical observations interpreted in the light of extensive basic research in animals implicate host resistance as a factor in the natural history and immunochemistry of cancer.

In 20 of 32 patients with terminal malignancy following immunization with homogenates of their tumor in Freund's adjuvant and hyaluronidase there was a significant antibody titer rise. The serologic titers were measured with the tanned sheep erythrocyte hemagglutinin method. Two steps were taken to prove the humoral nature and specificity of this antibody titer rise and its relation to the whole tumor material injected intramuscularly or intradermally. Cohen fractions 1, 2, and 3 were harvested and injected into or adjacent to subcutaneous metastatic tumor nodules which were subsequently biopsied and of which slides were prepared. This plasma protein fraction was cytotoxic to certain generations of the tumor cells. Second, the patients were skin tested and an immediate reaction was obtained with the whole tumor material; no reaction was obtained with the control material. It was concluded that a component of the cancer cell was antigenic and there was a humoral cytotoxic antibody produced against

the antigen. These findings may explain the unpredictable behavior of malignant tumors in different hosts. Case histories with the associated experimental data and clinical implications will be presented.

137. Effects of changing conductivity of the intracardiac blood on the electrocardiogram

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Introduced by Franklin D. Johnston, M.D.

Previous theoretical considerations and data from models have suggested that changes in the conductivity of the intracardiac blood produce changes in the surface electrocardiogram. Horan and co-workers have produced changes in the electrocardiogram by the intracardiac injection of carbon dioxide and have suggested that the alterations were secondary to changes in the intracardiac conductivity. In the present study, changes in intracardiac conductivity were recorded in the intact animal with the stimultaneously recorded electrocardiogram.

Five to 50 ml. of 5 per cent saline, 2 per cent potassium chloride, 50 per cent magnesium sulfate, Urokon, 5 per cent glucose, whole blood, or packed red cells in 5 per cent glucose were injected quickly into the right atrium or left ventricle of 9 anesthetized dogs. An electrocardiogram and the conductivity of the blood in the right and left ventricles were continuously recorded, the latter by means of a special conductivity catheter in each chamber. Cardiac output was measured by the indicator dilution technique.

Saline, magnesium sulfate, potassium chloride, and Urokon caused an increase in the conductivity of the intracardiac blood, packed red cells in 5 per cent glucose caused a decrease in blood conductivity, and 5 per cent glucose caused a less marked decrease. Whole blood injection caused no change in conductivity. There was a constant relationship between intracardiac conductivity and the electrocardiogram in 52 separate injections. An increase in conductivity of ventricular blood was consistently associated with an increase in the voltage recorded from activation of the ventricle and a decrease in the conductivity of ventricular blood was associated with a reduction in the voltage recorded from activation of that ventricle.

It is concluded that the observed electrocardiographic changes are due to changes in conductivity per se, and are independent of the chemical nature of the injectate and unrelated to pressure or positional changes induced by the injection. In the presence of radial ventricular activation in the normal dog, the results provide evidence to support the theoretical considerations of Brody. The method is applicable to the study of normal and abnormal ventricular activation in animals and man.

138. The relationship of acquired agammaglobulinemia to other mesenchymal diseases: A family study

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The problem of the relationship of the various collagen diseases to each other and to other diseases of the reticulum is one which has received increasing attention in recent years. A high incidence of collagen diseases among agammaglobulinemic patients has been a most provocative finding in this regard. A family of four generations in which the propositus has primary acquired agammaglobulinemia and rheumatoid arthritis and in which an extraordinary frequency of mesenchymal diseases occurred is presented. Among the 48 relatives of the propositus there are 2 cases of idiopathic thrombocytopenic

purpura, 1 case of well-documented fatal systemic lupus erythematosus, and 1 person with a fatal illness, poorly characterized, but compatible with diagnosis of either lymphoma or systemic lupus erythematosus. Three other members of the family have facial skin rashes, one of these with hemorrhagic tendency, and one other member has hyperproteinemia and hyperglobulinemia. Another has a mild hemorrhagic diathesis and cancer of the breast. Last, a ninth relative of the propositus had 2 attacks of rheumatic fever as a child.

Physical examination, paper electrophoresis, immunoelectrophoresis, and antinuclear antibody determinations were performed on 41 living members of this family. These studies revealed an asymptomatic member who has abnormalities of the immune globulin components as revealed by immunoelectrophoresis, consisting of decreased gamma globulins, and absent beta-$_{2M}$ and beta-$_{2A}$ proteins. This represents the syndrome described by Giddeon and Scheidegger in 1957.

An additional family was studied in which the propositus also suffered from rheumatoid arthritis and agammaglobulinemia. Numerous members of her family suffered from crippling rheumatoid arthritis. These families present a stimulating addition to the growing evidence that primary acquired agammaglobulinemia is indeed a hereditary disease, and that it may be etiologically related to several other diseases of the mesenchyme.

139. The acute pressor effect of parenteral cadmium and mercury ions

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Because the concentration of renal cadmium varies strikingly with the age and geographic origin of human subjects and because of our repeated but inconstant observation that chronic feeding of small doses of cadmium induces hypertension in rats, the vasoactivity of parenterally administered cadmium was studied. Direct measurement of arterial pressure consistently revealed significant hypertension following injections of cadmium or mercury into femoral arteries of normotensive Sprague-Dawley rats anesthetized with 30 mg. of pentobarbital per kilogram of body weight. In 12 rats weighing 200 grams, rapid injection of 10 μg (1/64 LD_{50}) of cadmium, as the chloride, dissolved in 0.5 ml. of isotonic saline produced a mean increase in systolic pressure of 38 ± 9 and in diastolic pressure of 30 ± 5 mm. Hg. For 40 μg of cadmium, the corresponding mean increases were 36 and 27 mm. Hg. The pressor effect was evident within ¼ minute, was maximal after 1 to 2 minutes, and was largely dissipated in 8 minutes, leaving the animals equally responsive to subsequent doses. The pressor effect was little affected by phentolamine, which blocks catechol amines, was gradually reversed by hydralazine, which probably acts directly on vessels, and was immediately obliterated by ethylenediaminetetraacetate, which chelates metals. Eighty micrograms of cadmium was strikingly depressor; 5 μg was usually inert.

Larger amounts of mercury produced similar but more marked hypertension. From 280 μg (¼ LD_{50}) to 70 μg of mercuric ion, as the sulfate, produced mean systolic increases greater than 70 and mean diastolic increases greater than 40 mm. Hg. For 18 μg of mercuric ion, the mean increases were 25 and 19 mm. Hg. Zinc was not vasoactive. Ten, 100, and 1,000 μg of lead, silver, copper, barium, and vanadium were tested; 100 μg of vanadium and 1,000 μg of silver, copper, and barium were pressor. Removing kidneys and adrenals from the circulation did not alter the pressor response. Concentrations of 10^{-6} molar mercuric ion invariably, and 10^{-4} cadmium ion occasionally, produced delayed and slow, but prolonged and marked, contraction of spirally cut strips of rabbit aorta, suggesting a direct vascular effect.

140. The action of potassium on the atrioventricular node in digitalized patients

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Introduced by Edward Massie, M.D.

Since the use of potassium in the treatment of digitalis intoxication has become popular, many consider potassium as a specific antagonist of digitalis. Previously it was shown that potassium infusions will increase the block at the atrioventricular node in patients with auricular fibrillation. Recently, dogs intoxicated with digitalis have been shown to develop various forms of atrioventricular block following potassium infusion. These observations suggested that potassium therapy may be contraindicated in patients whose primary manifestation of digitalis intoxication is a form of atrioventricular block. Because of these observations, and in order to establish the magnitude of synergistic action of potassium and digitalis on the atrioventricular node, patients with heart disease and sinus rhythm who were digitalized and on daily maintenance were given infusions of potassium chloride ranging from 10 to 20 mEq. in a period of 15 to 25 minutes.

Ten patients with various types of heart disease were studied. Control electrocardiograms and plasma potassium levels were obtained. The patient was monitored continuously by electrocardiogram; subsequent plasma potassium levels were obtained either when a significant increase in atrioventricular conduction occurred or at the end of the infusion.

Eight of 10 patients showed an increase in P-R interval in excess of 14.2 per cent of the control value. Six of 10 patients had increases in excess of 20 per cent of control values, and 1 patient developed 2:1 atrioventricular block. One patient had a 115 per cent increase over the control interval. Plasma potassium levels did not change significantly except in the one patient with the greatest increase in P-R interval. Changes in atrioventricular conduction rapidly subsided after the infusion was stopped.

The increase in atrioventricular conduction and the occurrence of 2:1 atrioventricular block in patients on maintenance digitalis are believed to be significant and demonstrate that potassium has a synergistic action with digitalis at the atrioventricular node. These observations suggest that potassium therapy for digitalis intoxication may be dangerous, if not contraindicated, when the major manifestation of intoxication is a form of atrioventricular block.

141. Continuously recorded changes in thoracic aortic blood flow in man in response to leg exercise

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In 5 normal men in the supine position, cardiac output was determined at intervals by sudden single injections of Coomassie blue into the superior vena cava, and thoracic aortic blood flow was recorded continuously for periods of 60 seconds by constant-rate injections of indocyanine green into the thoracic aorta (J. Appl. Physiol. 11: 405, 1957). Determinations were made at the following times: (1) with the subjects at rest; (2) while they started pedalling a bicycle ergometer and for the first 30 seconds thereafter; (3) before, during, and for 30 seconds after a 5 minute period of this exercise; and (4) at 5 and 10 minutes after start of the recovery period. Heart rate, mean aortic pressure, central venous pressure, and oxygen consumption were also measured.

Average oxygen consumption at rest was 254 (203 to 327) ml. per minute, increasing with exercise to 1,049 (710 to 1,491) ml. per minute. Thoracic aortic blood flow and heart

rate increased and lower body vascular resistance decreased immediately with the onset of exercise. By 10 seconds there was also a uniform temporary decrease in mean aortic pressure. After 25 seconds of exercise, thoracic aortic blood flow had increased to 189 (126 to 308) per cent and heart rate to 130 (111 to 144) per cent, and lower body vascular resistance had decreased to 58 (34 to 79) per cent of the resting values. With continuing exercise, further moderate changes occurred, and these values averaged 248 (143 to 362), 149 (127 to 177), and 41 (30 to 62) per cent, respectively, at 5 minutes, when total cardiac output had increased to 154 (130 to 180) per cent of control value. Thoracic aortic blood flow increased further and lower body vascular resistance decreased during the first 25 seconds of recovery. After 5 minutes of rest, all average values had approached or attained the control values, with only slight additional changes at 10 minutes.

These findings are in agreement with those obtained in unanesthetized dogs. They indicate that compensatory cardiovascular changes occur rapidly with the onset of exercise and that changes in heart rate, blood flow, and vascular resistance take place before the other circulatory alterations, most of the compensatory changes occurring within the first 30 seconds.

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		GOOD	FAIR	TRANSIENT	FAILURE	
Lymphoma	**74**	**34**	**3**	**5**	**23**	**9**
Hodgkin's Disease	29	10	3	4	9	3
Lymphosarcoma	21	15	0	0	3	3
Multiple Myeloma	16	9	0	0	4	3
Reticulum Cell Disease	8	0	0	1	7	0
Leukemia	**23**	**10**	**0**	**0**	**8**	**5**
Chronic Lymphatic Leukemia	8	4	0	0	3	1
Acute Monoblastic Leukemia	11	5	0	0	3	3
Acute Myeloblastic Leukemia	4	1	0	0	2	1
Carcinoma (Breast, Lung, and Solid Tumors)	**29**	**2**	**1**	**1**	**23**	**2**
Miscellaneous (Mycosis Fungoides, Psoriasis)	**4**	**0**	**0**	**1**	**3**	**0**
Total	130	46	4	7	57	16

Adapted from Wall, R. L., and Conrad, F. G.*

Note that the neoplastic disorders most responsive to Cytoxan were lymphosarcoma, multiple myeloma, Hodgkin's disease, and chronic lymphatic leukemia. Occasionally, good results were observed in acute monocytic leukemia and carcinoma of the breast.

Other advantages noted in this study*

• multiple routes of administration, permitting prolonged maintenance therapy • lack of latency period for bone marrow depression • failure to produce significant thrombocytopenia • potential therapeutic effect in diseases usually unresponsive to other mustard compounds (*e.g.*, myeloma).

*Wall, R. L., and Conrad, F. G.: Arch. Int. Med. *108*:456-482, 1961.

INDICATIONS: Cytoxan is valuable for palliative therapy of certain malignant neoplasms, particularly some of those arising in the reticuloendothelial and hematopoietic systems and certain solid tumors.

Types of cancer which have proved relatively more susceptible or more resistant to Cytoxan therapy may be grouped as follows:

Group I: Neoplasms relatively susceptible to Cytoxan
- Hodgkin's disease
- Lymphomas: lymphosarcoma; giant follicular lymphoma; reticulum cell sarcoma
- Leukemia: acute; chronic
- Mycosis fungoides

Group II: Neoplasms relatively resistant to Cytoxan
- Malignant neoplasms of the breast and the ovary*
- Malignant neoplasms of the lung, the gastrointestinal tract and the genitourinary system, including the cervix and the uterus
- Malignant neoplasms of miscellaneous origin
- Malignant melanomas

*Malignant tumors of these organs are somewhat more susceptible to Cytoxan therapy than are the others included in this group.

DOSAGE: *For neoplasms relatively susceptible to Cytoxan* — Patients with lymphomas and other neoplasms believed to be relatively susceptible to Cytoxan therapy are given an initial dose of 2 to 3 mg./Kg./day intravenously. White blood counts and platelet determinations should be made daily or twice weekly and the dosage adjusted accordingly. Intravenous infusions should be continued for at least 6 days unless otherwise indicated. A leukopenia of between 1500 and 5000 cells per cu. mm. (or lower) may be expected between the tenth and fourteenth day. In the presence of a leukopenia of less than 2000/cu. mm. Cytoxan should be discontinued until the white cell count returns to 2000 to 5000 (usually within a week). Dosage is subsequently adjusted as indicated by the patient's objective response and the leukocyte count. If the patient is subjectively improved, if the size of the tumor has decreased, or if the white cells are satisfactorily maintained between 2000 and 5000/cu. mm. oral dosage may be instituted equivalent to intravenous dosage.

Thrombocytopenia is rarely observed on this regimen. If platelet counts of less than 100,000/cu. mm. are observed, the patient should be watched carefully. If platelets continue to decrease, Cytoxan should be discontinued.

The patient who has had previous treatment with alkylating agents, or x-ray, or is debilitated may be more susceptible to bone marrow depression, and initial Cytoxan doses should be more conservative than the above. Such patients should have more frequent hematologic evaluation. Good medical practice demands access to a reliable hematologic laboratory when using Cytoxan.

For neoplasms relatively resistant to Cytoxan — Patients with carcinomas and other malignant neoplasms believed to be less susceptible to Cytoxan therapy are given a dose of 4 to 8 mg./Kg./day intravenously. Unless there are indications to the contrary, this dose is continued for 6 days, then stopped. Leukopenia usually ensues on the tenth to fourteenth day after the first dose of Cytoxan. Thrombocyte reduction is not common, and platelets may actually increase. The leukocyte count promptly returns toward normal levels in most cases, and as it begins to increase, sufficient Cytoxan is administered to maintain it near 2000 to 5000/cu. mm. This may be accomplished by two intravenous injections weekly, or by oral administration, or by a combination of both routes. An oral dosage of 50 to 200 mg. daily or an intravenous injection of 5 mg./Kg. twice weekly will usually suffice.

The platelet and leukocyte counts should be followed carefully, and the prior treatment history of patients carefully evaluated as delineated above.

Leukopenia as a guide to adequacy of dosage — The best objective measure for dosage seems to be the number of circulating white blood cells. This is used as an index of the activity of the hematopoietic system, especially the bone marrow. The mechanism by which Cytoxan causes a reduction in the level of white blood cells is not known, but cessation of dosage results in an increase in the level, indicating that the hematopoietic system had not been permanently affected. When large doses (8 mg./Kg./day for 6 days) are given initially, the white cell count falls rapidly. Following the cessation of the 6-day course, the white cells may continue to decline for as long as 8 days and then increase. The reduction of the white cell count during Cytoxan therapy and its subsequent increase when therapy is discontinued can be repeated in the same patient. Maximal reduction in leukocyte count indicates the maximal permissible Cytoxan level for therapeutic effect. Leukopenic patients must be watched carefully for evidence of infection.

Total white blood cell and thrombocyte counts should be obtained 2 or more times weekly in order to evaluate therapy and to adjust dosage.

SIDE EFFECTS: Although Cytoxan is related to nitrogen mustard, it has no vesicant effect on tissue. It does not traumatize the vein when injected intravenously, nor does it cause any localized tissue reaction following extravasation. It may be administered intravenously, intramuscularly, intraperitoneally, intrapleurally or directly into the tumor, when indicated. It is apparently active by each of these routes.

Nausea and vomiting are common and depend on dose and on individual susceptibility. However, many investigators accept the nausea and vomiting in favor of maintaining maximal therapy. The vomiting can be controlled with antiemetic agents.

Alopecia is a frequent side reaction to Cytoxan therapy. It has been observed in 28% of the patients studied in this country. The incidence is greater with larger doses. The loss of hair may first be noted about the 21st day of therapy and may proceed to alopecia totalis. This effect is reversed following discontinuance of Cytoxan; during reduced maintenance therapy, hair may reappear. It is essential to advise the patient in advance concerning this effect of the drug.

Dizziness of short duration and of minor degree has occasionally been reported.

Leukopenia is an expected effect and can be used as a guide to therapy. Thrombocytopenia may occur, especially after large doses. The leukocyte or platelet counts of an occasional patient may fall precipitously after even small doses of Cytoxan, as with all alkylating agents. The drug should be discontinued in such patients and reinstituted later at lower dosage after satisfactory hematologic recovery has occurred. Prior treatment with x-ray or with other chemotherapeutic agents frequently causes an earlier or exaggerated leukopenia or thrombocytopenia after Cytoxan medication. Only rarely has there been a report of erythrocyte or hemoglobin reduction.

ADMINISTRATION: Add 5 cc. sterile water (Water for Injection, U.S.P.) to 100 mg. of Cytoxan in the sterile vial (add 10 cc. to 200 mg. vial). Shake, allow to stand until clear, remove with sterile syringe and needle and inject.

The freshly prepared solution of Cytoxan may be administered intravenously, intramuscularly, intraperitoneally, intrapleurally, or directly into the tumor. The solution should be administered promptly after being made but is satisfactory for use for three hours after preparation.

If the patient is receiving a parenteral infusion, the Cytoxan solution may be injected into the rubber tubing if the solution is glucose or saline.

No thrombosis or thrombophlebitis has been reported from injections of Cytoxan. Extravasation of the drug into the subcutaneous tissues does not result in local reactions.

PRECAUTIONS: Cytoxan should not be given to any person with a severe leukopenia, thrombocytopenia, or bone marrow infiltrated with malignant cells. It may be given with suitable precautions to patients who have had recent x-ray treatment, recent treatment with a cytotoxic agent, a surgical procedure within 2 to 3 weeks, or debilitated patients.

AVAILABILITY: Cytoxan is available as follows:

Cytoxan for Injection, 100 mg., a sterile dry-filled vial containing 100 mg. cyclophosphamide and 45 mg. sodium chloride. Packaged, 12 vials per carton.

Cytoxan for Injection, 200 mg., a sterile dry-filled vial containing 200 mg. cyclophosphamide and 90 mg. sodium chloride. Packaged, 12 vials per carton.

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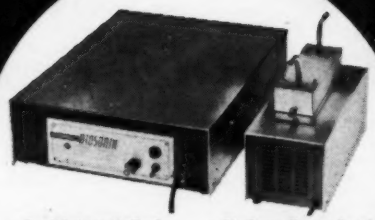

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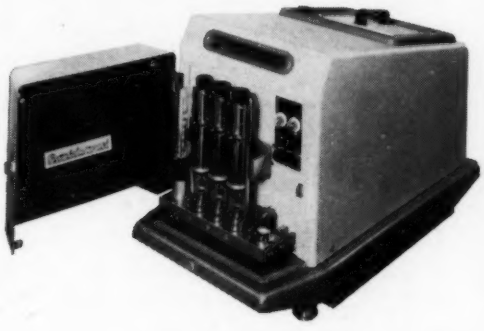

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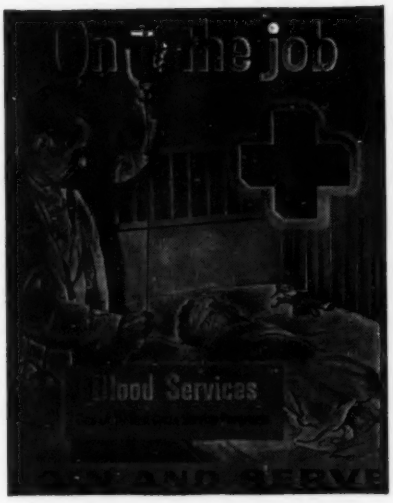

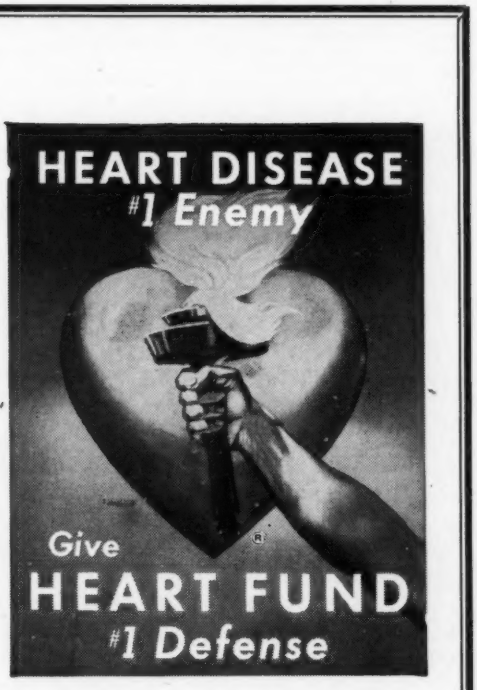

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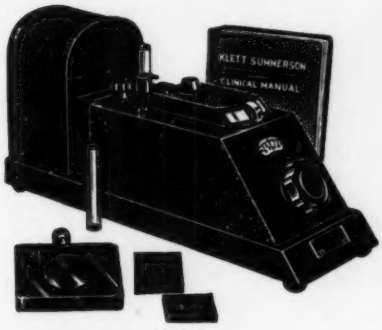

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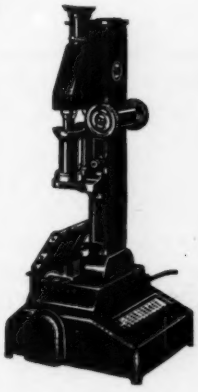

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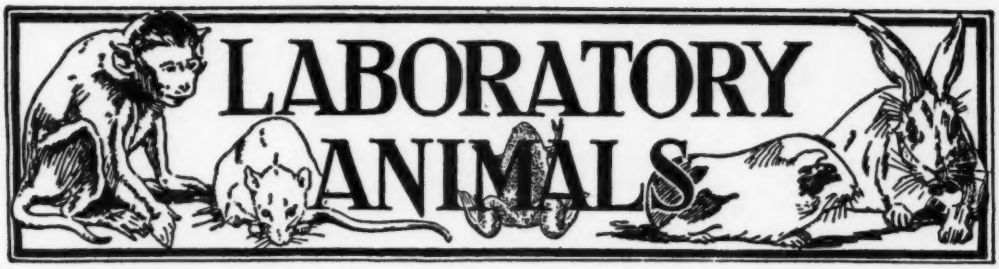

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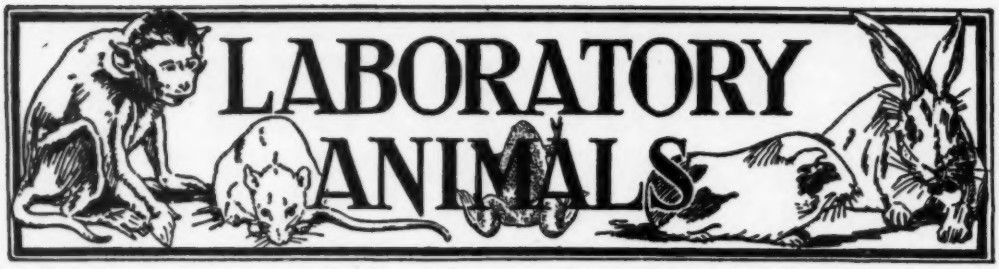

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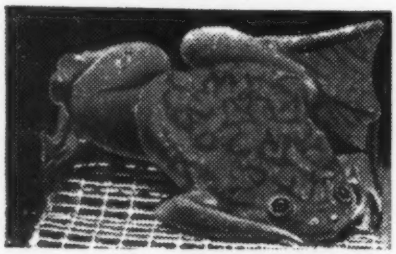

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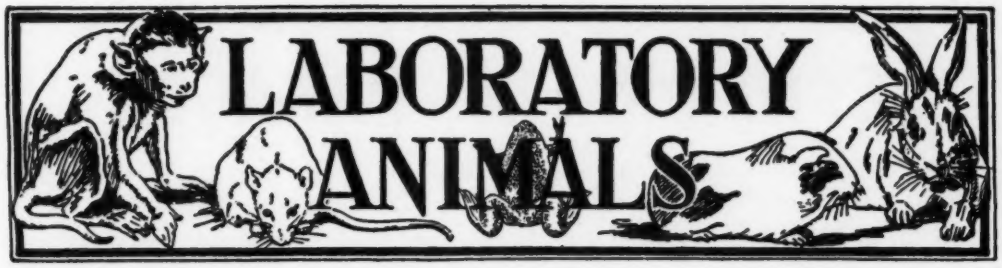

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*Bauer, A. C. and Redner, W. J., Jr., Obst. & Gynec. 17:325, 1961

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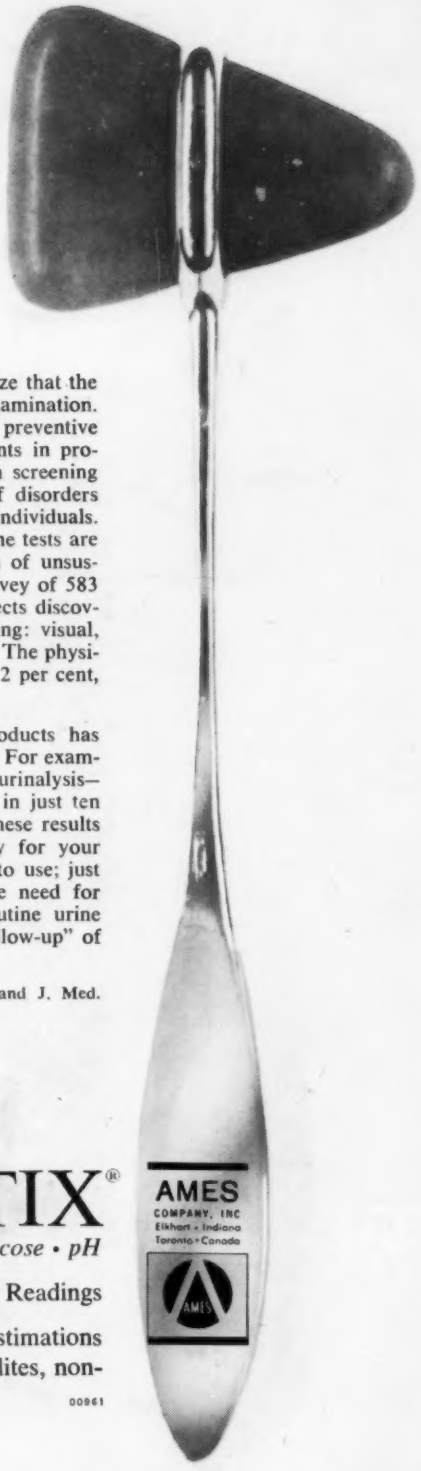

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*McCombs, R. P., and Finn, J. J., Jr.: New England J. Med. *248*:165 (Jan. 29) 1953.

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